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THE ACTION OF WATER MOCCASIN VENOM ON THE ISOLATED FROG HEART

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The action of various snake venoms on isolated frogs hearts has been studied by many investigators. Cobra venom has been so studied by Elliott (1901), Gunn (1912), Cushney and Yagi (1918), Gunn and Heathcote (1921), Meurling (1935), and others. Magenta (1922) studied the effects produced by venoms from fifteen species of poisonous snakes, by the Straub method. Unfortunately his results are published in such abbreviated form that the effects of individual venoms cannot be determined.

Among the venoms Magenta used was that of the water moccasin, Agkistrodon piscivorus Lacépède. The action of the venom of this species of snake has also been studied by Essex and Markowitz (1930), and by Essex (1932), using hearts of rabbits. When a Ringer-Locke perfusion fluid to which dried venom was added to make a final dilution of 1:250,000 to 1:500,000 was used, the hearts were incapacitated in ten to fifteen minutes, indicating a much greater cardiac toxicity than is possessed by the venom of the timber rattlesnake, Crotalus horridus. In view of the small amount of study of the water moccasin venom, the author determined to investigate the venom, especially because of the frequent statement that this venom is essentially similar to that of the North American rattlesnakes, e.g., Noguchi (1909), Essex and Markowitz (1930), Essex (1932).

In all experiments, isolated hearts of spring or summer frogs were used. A modified Straub method, "constant drip," was necessary. The modifications were: 1, arrangement of the reservoir containing the perfusion fluid so that a constant stream of fluid ran into the cannula; 2, a cotton wick at the upper end of the cannula, which prevented formation of a

large meniscus, with consequent alteration of the diastolic filling; it also distributed the overflow evenly over the exterior of the heart. The rate of flow of the perfusion fluid was such that one or two drops were expelled at each systole.

The venom was collected in the latter part of the summer of 1938, by the well-known method of "milking." The collected venom was dried under an electric fan, ground in a mortar and the various batches mixed to form the stock dry venom. The stock represented ten milkings from twenty-eight snakes. Mitchell and Reichert (1886), and Macht (1933 and 1937) have shown that any alteration in such dried venoms is in the direction of some loss in potency, if there be any change.

The perfusion fluid used in all experiments was Ringer solution of the following composition, expressed in millimoles per liter: NaCl 112.20, KCl 1.88, $CaCl_2$ 1.04, $NaHCO_3$ 2.38. To this was added enough weighed dry venom to give the desired dilution.

The contractions of the hearts were recorded on a kymograph by means of a light heart lever, fastened to the tip of the ventricle by a fine silk thread. For each dilution used eight[1] hearts were run. In the control group of eight hearts, one failed completely in 180 minutes. This duration was considered as maximum in all subsequent experiments. In experiments with venom, the hearts were run in the Ringer solution until they had become physiologically constant; at this time the cannula was filled with the Ringer-venom solution and the drip started.

Each record was measured for amplitude of contraction. For each, the initial amplitude was arbitrarily considered as ten centimeters, an approximation of the actual average amplitude. These results were averaged for each experimental group, i.e., controls, 1:1,000, 1:10,000, 1:100,000, and 1:1,000,000 venom-Ringer solutions. The results are shown as figure 1.

The control hearts run with Ringer solution showed no stimulation and a decrease in amplitude of 43 per cent in 180 minutes; the hearts subjected to 1:1,000 venom in Ringer showed practical standstill in 18 minutes and no stimulation throughout the experiment. When venom was used in a concentration of 1:10,000 there was a brief period of increased amplitude (maximum: 43 per cent; duration: 7 minutes) succeeded by rapid decrease to zero in 24 minutes. The solution of venom 1:100,000 produced a maximum increase of 8 per cent; the duration of the stimulating action was 17 minutes absolute or 43 minutes when compared with the controls. Hearts run with 1:1,000,000 venom-Ringer solution showed an average maximum stimulation of 11.4 per cent and remained more effective than the controls throughout the entire 180 minutes.

[1] The group run in 1:100,000 venom consisted of seven hearts.

The effects on heart rate of the Ringer solution and the various venom solutions are shown in figure 2. The rate of beating of the controls remained rather uniform, i.e., O. Frank's law was applicable, the changes

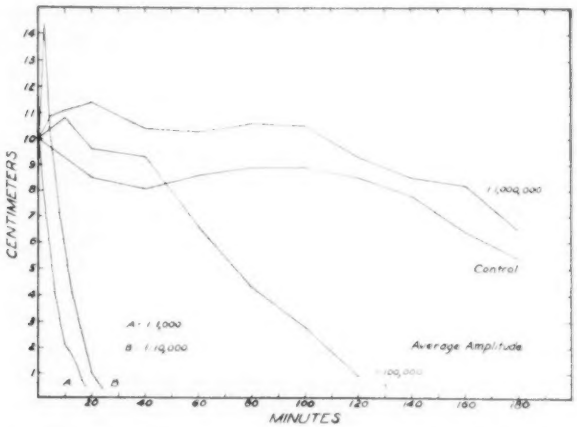

Fig. 1. Average contraction amplitudes of control hearts and those subjected to venom-Ringer solution.

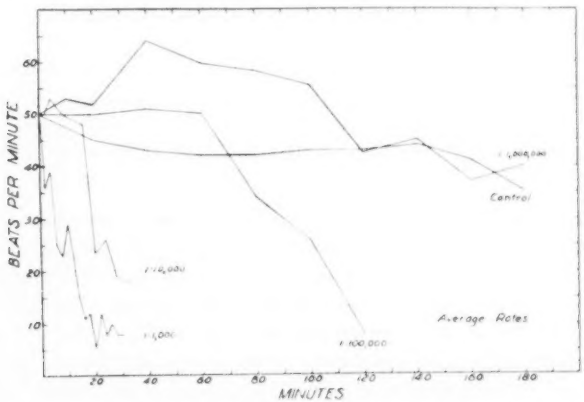

Fig. 2. Average rates of control hearts and those subjected to venom-Ringer solution.

in rate being in general inversely proportional to the changes in amplitude. In 180 minutes the rate of the controls decreased 35 per cent. Marked slowing or complete stoppage resulted from the application of 1:1,000 venom solutions; partial recovery followed. With 1:10,000 venom solutions

there was first marked slowing, followed by a slight increase in rate; this in turn gave way to rapid failure of contraction. The original slowing could be abolished by atropine. In 1:100,000 venom solutions, three of the hearts showed initial slowing, three initial stimulation, and one showed rapid failure with no recovery. The effect of averaging the results is to obscure these effects on the graph. Hearts treated with 1:1,000,000 venom solution showed in several instances very slight initial slowing. There followed great increases in rate in three hearts, moderate increases in three, and in two there was little change in rate.

Shown in figure 3 are the effects of the various concentrations of venom on the output of the hearts; the output of the controls is included. The curves are the products of the amplitude from figure 1 and the rate from figure 2. The measure is relative, but agrees well with several experi-

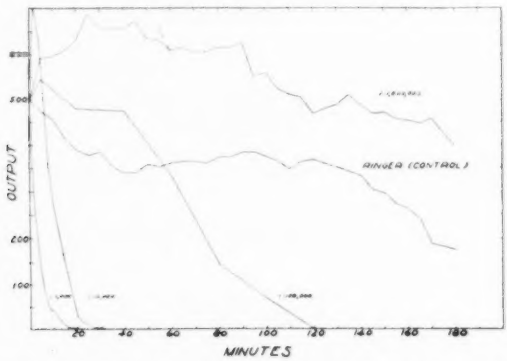

Fig. 3. Average output of control hearts and those subjected to venom-Ringer solution.

ments where actual volume changes were measured. Because of the reciprocal relationship between amplitude and rate, the product of the two is a more reliable measure of effect than either alone. The output of the 1:1,000 hearts fell to zero in 20 minutes; that of the 1:10,000 hearts in 32 minutes and that of the 1:100,000 hearts in 120 minutes. The control hearts showed a 65 per cent loss of efficiency in three hours; the hearts treated with 1:1,000,000 venom solution suffered a loss of only 20 per cent in the same time. The first two solutions were severely cardiotoxic; the 1:1,000,000 solution was beneficial; the 1:100,000 solution produced stimulation, followed by rapid failure.

The order of failure in the hearts was first the ventricle, then the atria, last the sinus venosus. The ventricle stopped in complete contraction in most of the hearts. In those treated with high dilutions of venom the

contraction was sometimes incomplete; mechanical stimulation induced complete contraction. The muscle became whitish and opaque, with a "cooked" appearance. The atria failed in wide dilatation. They were also opaque. The sinus venosus remained clear and beat for some time after failure of the atria and ventricle.

Dissociation occurred only rarely except in the terminal stage of ventricular activity, where complete ventricular failure was imminent. This is in contradiction to Magenta's (1922) results. When dissociation did occur, it was of the type of dissociation with capture.

Extra systoles were rather common. Muscular irregularities frequently occurred, accompanied by local regions of contracture in the ventricle. These effects disappeared as the elasticity of the myocardium decreased, a constant effect with all venom solutions except the weakest, as shown by progressive failure of rebound of the heart lever.

The venom acted upon the vagal endings or the ganglion cells of the heart to produce slowing. This slowing could be almost completely abolished by atropine. In several of the hearts, especially those treated with high dilutions of the venom, the release from vagal effects was quite sudden; in one case the rate doubled in less than one minute. The probable site of action is the ganglion cells, which are first stimulated, then depressed. This is in accordance with the stimulation and subsequent depression of the myocardium seen above.

An outstanding effect of the venom solutions was the tremendous increase in the permeability of the atria. These became so permeable that the "constant drip" method was necessary to keep the Straub cannula filled. Obviously the endocardium was severely damaged. There appeared to be no fluid loss through the ventricle, probably because the much heavier, more dense muscle prevented fluid escape.

Because of the many obvious muscular effects and the constant dialyzing action of the "constant drip," the author believes the results to be due to direct action of the venom on the myocardium, endocardium, and intrinsic ganglia, rather than to the action of lysolecithin and histamine produced by the action of the venom on the tissues. This view is in agreement with that of Essex (1932) and receives correlative support from the work on rattlesnake (Crotalus horridus) venom of Dunn (1934); Essex and Markowitz (1930b); Dragstedt, Mead, and Eyer (1938); and from the demonstration of such direct effects of snake venoms on protoplasm by Lepow (1938) and the importance of the change of lecithin into lysolecithin on the properties of surface films as shown by Hughes (1935). The hypothesis that the effects of the venom are caused by the lysolecithin and histamine produced, as stated by Belfanti (1925), Magenta (1922), Houssay (1930), and Houssay, Negrete, and Mazzocco (1933)

seems unnecessarily complex. This aspect of the problem is well reviewed by Kellaway (1939). The author believes the venom acts directly and that the lysolecithin and histamine are by-products of such direct action, rather than being the causative agents of the tissue changes.

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SUMMARY

The venom of the water moccasin, Agkistrodon piscivorus Lacépède, acts directly on the endocardium, myocardium and intrinsic ganglia of the isolated frog heart.

In 1:1,000 concentration, the venom has a depressant action on the heart; in 1:10,000 and 1:100,000 concentrations there is preliminary stimulation, followed by failure.

Concentrations of 1:1,000,000 are stimulating; the hearts exceeded the efficiency of the controls throughout the experimental period.

The ventricle failed first; stoppage was usually in complete contraction, although with weak venom solutions it might be in partial contraction. There were muscular irregularities and contractures with all but the weakest solutions.

The atria were rendered highly permeable, permitting rapid escape of fluid through their walls. Failure was in extreme dilatation, occurring after that of the ventricle.

The sinus venosus appeared to be quite refractory to the venom action and failed last.

Dissociation rarely occurred, except in terminal period of ventricular action, where it presaged complete failure in short order.

Stronger venom solutions slow the heart initially by action on the vagus mechanism, probably the ganglion cells. This slowing can be almost completely abolished by atropine.

After failure both the atria and ventricle were opaque and whitish; they looked "cooked."

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THE PRESSOR RESPONSE TO ADRENALIN IN THE COURSE OF TRAUMATIC SHOCK

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The theory that hypersecretion of adrenalin is responsible for the fall in blood pressure in traumatic shock, first proposed by Bainbridge and Trevan (1), has not been widely accepted. In recent years, this theory has been revived by Freeman (2) and Cannon (3). Since Lorber, Kabat and Welte (4) have confirmed the importance of the nervous factor in traumatic shock, it seemed possible that reflex hyperactivity of the sympathetic nervous system might be the mechanism by means of which afferent nerve impulses produce shock. The realization that the evidence supporting the adrenalin theory was of an indirect character, not having involved experiments on animals in shock, prompted an investigation of the rôle of adrenalin in shock.

I. *Traumatic shock resulting primarily from afferent nerve impulses.* A simple technique was devised to produce traumatic shock, involving primarily the nervous factor. Cats were anesthetized with chloralose in doses of 80 mgm. per kilogram of body weight administered intravenously. The hair on the hind legs and lower abdomen was removed and these regions were tightly wrapped with adhesive tape. The leg to be traumatized was bandaged with two layers of adhesive tape and, following trauma, another layer of tape was added. The pelvis was bandaged to the level of the fifth lumbar vertebra. The leg was then maintained in moderate elevation to facilitate venous return. By these simple procedures, it was possible to minimize local fluid loss in the traumatized limb. Blood pressure was recorded continuously on a kymograph by means of a mercury manometer connected to a cannula in the common carotid artery. Chlorazol fast pink, injected into the tubing just above the cannula in 0.5 cc. quantities every half hour, was used as the anticoagulant (5). In all experiments, tracheotomy was performed and the airway inspected repeatedly to be certain that it was clear.

Trauma consisted of 100 blows to the hind leg, which were sufficient to produce multiple fractures of the long bones. The course of the changes in blood pressure following trauma is illustrated in figures 1 and 2. In most instances, the blood pressure rose sharply during trauma. Following

this, the blood pressure fell in every experiment, finally resulting in the death of the animal. In eight experiments, the average survival following leg injury was 2½ hours, while the range of survival was 70 minutes to longer than 7 hours. After termination of the experiment and an autopsy, the tape was removed and the hind quarters carefully separated and weighed to determine the local fluid loss (6). The average fluid loss in the traumatized limb was 0.65 per cent of the body weight with a range from zero to 1.85 per cent of the body weight. There was no significant relation between the magnitude of the fluid loss and the duration of survival. The loss of fluid was small enough in relation to the total blood volume in most instances so that the local fluid loss could be considered of little significance in the etiology of the shock in these experiments (7).

In order to be certain that the nerve impulses from the traumatized limb constituted the essential factor in the production of shock, three control experiments were performed. Fluid loss was minimized by bandaging the limbs with tape, while the nervous factor was eliminated by acute section of the spinal cord at the level of the first lumbar vertebra. Trauma in these animals failed to produce shock or even a significant fall in blood pressure. In one cat, the blood pressure initially was 131 mm. Hg and eight hours after trauma, at which time the experiment was terminated, was 122 mm. Hg. In another experiment, the blood pressure at the beginning was 142 mm. Hg and the final blood pressure seven hours after trauma was 120 mm. Hg. In the third cat, blood pressure at the start was 132 mm. Hg and 4¼ hours later was 136 mm. Hg, when the pressure fell suddenly to zero and the animal died of massive hemorrhage at the site of the cord section. These experiments indicate that toxic factors are not paramount in the etiology of this type of traumatic shock and that elimination of significant local fluid loss and of nerve impulses from the site of injury prevents the development of shock.

It has also been possible to confirm the results of Lorber, Kabat and Welte (4), who emphasized the relationship of the depth of general anesthesia to the susceptibility of the animal to traumatic shock. In this experiment, only 33 mgm. per kilogram of chloralose was administered. After a fairly rapid fall of blood pressure to shock level, the pressure rose gradually but steadily over a number of hours. About four hours after trauma, an additional 33 mgm./ kilo of chloralose was administered intravenously, and from then on the blood pressure fell rapidly and the cat expired within an hour. The fluid loss into the traumatized limb was negligible. Since the total dose of chloralose in this experiment was still below the usual anesthetic dose (only 67 mgm./kilo), one can only conclude that the administration of the anesthetic following trauma greatly increased the susceptibility of the animal to traumatic shock.

II. *Pressor effects of physiological doses of adrenalin in the course of shock.*

In eight cats under chloralose, with fluid loss minimized as described above, adrenalin was injected intravenously about every fifteen minutes before and after trauma to the hind leg. The external jugular vein was cannulated and connected to a burette containing warm Ringer's solution. Adrenalin in doses of 0.005 mgm. or 1/20 cc. of 1:10,000 solution was in-

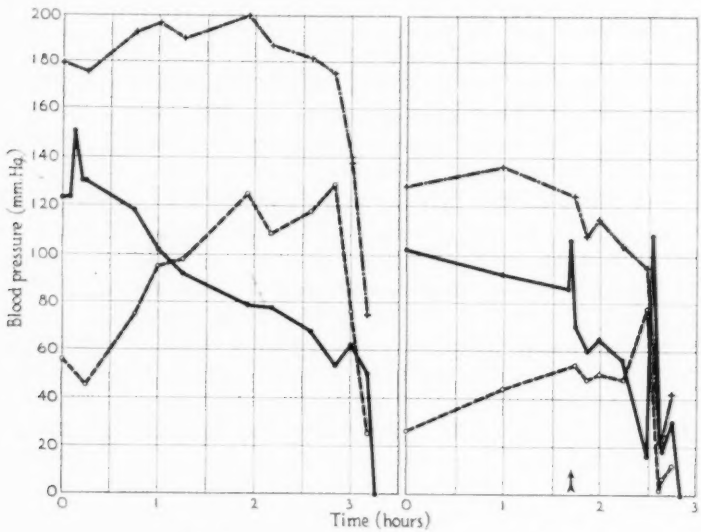

Fig. 1 Fig. 2

Fig. 1. Influence of adrenalin on traumatic shock (expt. 4). The solid line represents the effects of trauma on the blood pressure. The lower broken line represents the rise in blood pressure produced by intravenous injection of 0.005 mgm. of adrenalin at regular intervals. The upper broken line with crosses represents the level to which adrenalin elevated the blood pressure. The arrow indicates the time of trauma. Fluid loss in the injured limb was 0.8 per cent of the body weight.

Fig. 2. Influence of adrenalin on traumatic shock (expt. 7). The solid line represents the effects of trauma on the blood pressure. The lower broken line represents the rise in blood pressure produced by intravenous injection of 0.005 mgm. of adrenalin at regular intervals. The upper broken line with crosses represents the level to which adrenalin elevates the blood pressure. The arrow indicates the time of trauma. Fluid loss in the injured limb was 0.5 per cent of the body weight. The marked rise in blood pressure before death was spontaneous.

jected into the rubber tubing just above the cannula with a 1 cc. tuberculin syringe and was then washed in with 5 cc. of Ringer's solution.

The most striking finding was the sudden marked decrease or complete disappearance of responsiveness to adrenalin just before death. This was observed in every experiment which resulted in fatal shock (figs. 1, 2). This marked decrease in responsiveness to adrenalin was usually evident

within 12 minutes of a good response to the drug and in one case was seen six minutes after an excellent response. The cats died 2 to 14 minutes

TABLE 1

Terminal loss of responsiveness to adrenalin

EXPERIMENT	TIME	BLOOD PRESSURE BEFORE	BLOOD PRESSURE DURING ADRENALIN	RISE IN BLOOD PRESSURE	REMARKS
1	2:27	77	108	31	
	2:45	62	62	0	
	2:55	0			Death
2	3:15	92	131	39	
	3:30	88	108	20	
	3:40	56	56	0	
	3:45	0			Death
3	10:11	60	106	46	
	10:20	66	66	0	
	10:24	0			Death
4	4:35	54	175	129	
	4:45	62	138	76	
	4:55	50	75	25	
	5:00	0			Death
6	6:27	85	153	68	
	6:40	75	75	0	Blood pressure continues to fall
	6:41	58	Less	0	
	6:42	0			Death
7	4:45	16	95	79	
	4:46	42			Spontaneous increase in blood pressure
	4:48	108			Marked retraction of n.m. and dilatation of pupils
	4:51	22	24	2	
	4:52	18	25	7	
	4:58	30	42	12	
	5:05	0			Death
8	7:47	108	152	44	
	8:07	108	143	35	
	8:20	79	99	20	
	8:23	0			Death

after the failure of adrenalin to elevate the blood pressure. The data on this terminal loss of responsiveness to adrenalin are presented in table 1.

It is interesting to note that the level of blood pressure at the time of failure of the adrenalin response varied from 75 to 22 mm. Hg. Another indication that the level of blood pressure did not determine the responsiveness to adrenalin is the observation that an excellent response to the drug was obtained at a blood pressure of 16 mm. Hg while a significantly decreased response was noted at a pressure of 88 mm. Hg.

A point of considerable interest is the fact that a slight or marked spontaneous rise in blood pressure immediately preceded the sudden decrease in responsiveness to adrenalin in some cases (figs. 1, 2). For some time before this, the blood pressure had been falling rather steadily. This sudden brief terminal rise of blood pressure was noted in three of the seven experiments which terminated fatally (3, 4, 7, table 1). The nictitating membranes, which had been quite relaxed throughout the post-traumatic period, contracted completely at the time of this brief rise of blood pressure. The pupils were also observed to become widely dilated at this time. The nictitating membranes then remained completely contracted and the pupils dilated until the cat expired. Terminal contraction of the nictitating membranes and dilatation of the pupils was a feature of all cases of fatal shock.

Excluding the terminal failure of adrenalin to affect the blood pressure, the magnitude of the elevation of blood pressure resulting from adrenalin administration was greater during shock than before trauma. As the blood pressure fell with developing shock, the rise, induced by adrenalin, became progressively more marked (fig. 1). Also, the more rapid the fall of blood pressure, the sharper the increase in magnitude of the adrenalin response (figs. 1, 2). In cases where there were significant fluctuations in the blood pressure record following trauma, the changes in the adrenalin response generally bore a reciprocal relation to the changes in blood pressure.

An examination of the absolute level to which the blood pressure rose as a result of adrenalin administration is also of interest. In two experiments, the level to which adrenalin elevated the blood pressure was higher for a time during shock than before trauma (fig. 1). In two other experiments, the level of adrenalin elevation fell sharply soon after trauma and was far below the pre-traumatic level throughout shock. In still other instances, the level of blood pressure attained following adrenalin injection decreased gradually and to a moderate extent in the course of shock (fig. 2). There was also a definite tendency for fluctuations in blood pressure to be accompanied by fluctuations in the level of adrenalin elevation in the same direction (fig. 2).

Discussion. Cannon (3) proposed that hyperactivity of the sympathetico-adrenal system excited by sensory nerve impulses is an important factor in traumatic shock. This theory was based in part on the well known clinical observation that cold, pain and fear, all of which have been

shown to stimulate the sympathetic nervous system, tend to intensify shock following trauma. It was also based on experiments which indicated that hyperadrenalinemia or continuous strong stimulation of the sympathetic system was capable of decreasing blood volume and lowering blood pressure significantly. It was assumed that prolonged, marked arteriolar constriction causes shock as a result of fluid loss on the basis of capillary anoxia and consequent increased permeability.

While earlier experimenters had succeeded in producing fatal shock by injections of this hormone, they used massive doses beyond the physiological range. For example, Erlanger and Gasser (8) working on dogs, injected 6 to 11 mgm. of adrenalin in 21 to 29 minutes and repeated this several times. This greatly exceeds the 0.0032 to 0.0037 mgm. per kilogram per minute reported by Cannon and Rapport (9) to be the maximum physiological secretion of adrenalin in response to afferent stimulation.

Freeman (2) studied the effects of continuous injection of physiological amounts of adrenalin on blood volume and blood pressure. Continuous intravenous administration of 0.001 to 0.006 mgm. per kilogram per minute of adrenalin to cats under dial over a period of two hours resulted in a rise in blood pressure at first from 140 to 210 mm. Hg followed by a fall to 180 mm. Hg. After stopping the injection, the arterial pressure fell to 100 mm. Hg. The average decrease in blood volume, measured by means of brilliant vital red in nine cats subjected to continuous intravenous adrenalin was 14 per cent. On the other hand, after ergotoxine, adrenalin injection no longer affected the blood volume.

These experiments do not constitute convincing evidence that hyperadrenalinemia may play a rôle in the production of traumatic shock. The decrease of blood volume reported is quite small. Moreover, it must be pointed out that during the injection of adrenalin the blood pressure remained higher than normal and only fell, and to a moderate extent, following withdrawal of the hormone. Also, results of a similar investigation by Hamlin and Gregersen (10) are contradictory to those of Freeman. Continuous injection of adrenalin in unanesthetized cats increased the blood volume. Furthermore, nembutal anesthesia resulted in a 10 per cent increase of blood volume. While adrenalin administration to cats under nembutal produced a decrease in blood volume, the volume did not fall below the pre-anesthetic level. In an ingenious experiment, Prohaska, Harms and Dragstedt (11) failed to produce shock by continuous intravenous injection of physiological quantities of adrenalin for as long as two weeks in unanesthetized dogs.

There remains the evidence presented by Freeman (2) that physiological hyperactivity of the sympathetico-adrenal system results in a significant decrease of blood volume and blood pressure. Hyperactivity of the sympathetic nervous system was produced by acute decortication of cats, resulting in "sham rage". In eight experiments, the blood pressure fell

from 137 to 55 mm. Hg in 90 minutes. The average decrease of blood volume in fifteen experiments was 21.9 per cent. This fall of blood pressure and blood volume in the pseudo-affective state was prevented by preliminary sympathectomy or administration of ergotoxine.

Our own experiments on the pressor effects of adrenalin in traumatic shock provide no support to the sympathetic hyperactivity theory. One would expect that if a persistent marked vasoconstriction were present during shock, the influence of adrenalin on the blood pressure would be very greatly reduced or absent. On the contrary, during almost the entire post-traumatic period, the rise of blood pressure resulting from adrenalin was greater than normal. The view that sympathetic hyperactivity is not a feature of shock produced primarily by afferent nerve impulses (except terminally) is also supported by the observation that the nictitating membranes were quite relaxed and the pupils of normal diameter during the course of shock. This does not rule out the possibility that sympathetic hyperactivity may play a rôle in shock following hemorrhage or fluid loss (12, 13).

SUMMARY

1. Trauma to a hind limb in which local fluid loss was minimized resulted in fatal shock in 2½ hours.

2. Shock was effectively prevented in such experiments by preliminary transection of the upper lumbar spinal cord.

3. Adrenalin produced a greater rise in blood pressure during shock than before trauma, except just before death, when the response of blood pressure to adrenalin suddenly disappeared.

4. No evidence was found to support the theory that hyperactivity of the sympathetic nervous system is responsible for the type of shock resulting primarily from afferent nerve impulses.

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AN ANALYSIS OF THE EXCITATORY AND INHIBITORY EFFECTS OF SYMPATHETIC NERVE IMPULSES AND ADRENALINE ON VISCERAL SMOOTH MUSCLE

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Stimulation of sympathetic nerves diminishes or stops the spontaneous movements of some visceral organs. For an understanding of this inhibitory effect it is important to know the changes in the functional state of visceral muscles produced by the nerve impulses. Experiments relating to this question, performed mainly on uterine muscle from the cat, will be reported in this paper.

A complication was introduced into these studies by the observation that the responses of the uterus to nerve stimulation were often diphasic, the inhibition being preceded by an excitatory action. Such responses have occasionally been observed before, but a more careful analysis suggested that they are the general type of response of the uterus to nerve stimulation.

No particular significance has hitherto been attached to such diphasic responses because it was accepted that the hypogastric nerve contains motor as well as inhibitory fibers. Various observations made it improbable, however, that the diphasic responses are due to specific excitatory and inhibitory fibers in the extrinsic nerves of the viscera. An attempt will be made in this paper to explain the experimental facts on the assumption that excitation and inhibition are two phases of the action of the same nerve impulses and that the character of the muscular responses, the preponderance of any one of these phases, is determined by the functional condition of the muscle.

METHODS. Cats under nembutal anesthesia were used in most experiments. Because uterine muscle is excitable electrically only during estrus (1), the animals received injections of theelin (500 int. units in oil at intervals of two days) for a few days before the experiment. One uterine horn was freed from the surrounding tissue and attached to an isometric lever. A firm ligature was tied around the other horn close to the body of the uterus. For the direct stimulation of the uterus, condenser discharges (7 μ F) were used. Cotton wicks soaked in Ringer's solution served as stimulating electrodes.

Uterine and intestinal strips were prepared as described previously (1). The contractions of the small intestine of the cat and dog were recorded by a balloon and mercury manometer.

RESULTS. Theoretically it would seem possible that inhibition of an automatically contracting organ can be produced by two changes within the muscle: 1, an interference with the mechanism responsible for the initiation of impulses; 2, a decrease in excitability, blocking muscular conduction. These possibilities will be tested in the experiments described below.

A. *Excitability.* In the non-pregnant cat, stimulation of the hypogastric nerve, or adrenaline injected intravenously, completely inhibits the motility of the uterus (cf. 10, 17). During inhibition the electric excitability as determined by the threshold for single electric shocks was

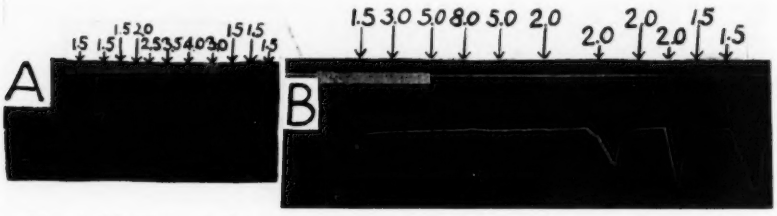

Fig. 1. Motility of the cat's uterus recorded by an isometric lever. During contraction, downward movement of the lever. Signal: stimulation of the hypogastric nerve (frequency about 20 per sec.). The excitability of the uterus was tested by stimulating the muscle directly with single condenser discharges (indicated by arrows). The numbers on the arrows are the voltages applied. Long arrows indicate ineffective stimuli. In B, the uterus became completely non-excitable during inhibition, in A weak responses were still elicited by strong stimuli. The experiments show a gradual rise in excitability following the cessation of nerve stimulation.

diminished or completely abolished (25 observations on 8 cats, fig. 1, A, B). Also the excitability for mechanical stimuli, tested by tapping the uterus with a blunt instrument, disappeared during stimulation of the hypogastric nerve.

These observations were confirmed in experiments on isolated uterine muscle. By the application of a few drops of adrenaline 1:500,000 the excitability of uterine strips, taken from animals in incomplete estrus (theelin injections for 4 days), was completely abolished and returned to normal within about 10 minutes. If the strips were taken from animals in full estrus (obtained by injections of theelin for 5 days) excitability was also diminished by adrenaline, but not completely abolished.

Similar results were obtained for intestinal muscle. Strips of the rabbit's small intestine which had been mounted in a moist chamber one-half to

one hour previously, were used. Such preparations, after they had become quiescent, responded to electric shocks by a contraction conducted over the entire muscle (2). Adrenaline $1:10^6$ raised the threshold 3 to 4 times and blocked conduction. Strong electric shocks still elicited local responses near the cathode.

In the isolated ureter of the cat and guinea pig, on the other hand, adrenaline increased electric excitability. Confirming previous observations (9), adrenaline also speeded up the conduction of peristaltic waves and often induced contractions.

In uterine muscle the effect of adrenaline varies in different species and under different physiological conditions. During estrus, adrenaline raised the threshold in guinea pigs only in about half the animals tested. The preparations never became non-excitable and in some there was no detectable effect. In two out of the ten animals tested, adrenaline produced a weak contraction. In the rabbit and in the pregnant cat a strong contraction was always produced under the same condition. These species differences run closely parallel with the well known differences in the responses to sympathetic nerve impulses (cf. 10, 16).

It becomes evident from these facts that adrenaline imitates both the excitatory and inhibitory effects of sympathetic nerve impulses. Observations which will be reported below confirm this conclusion.

B. *Diphasic responses.* In the cat's uterus a weak contraction preceding inhibition was previously found during the early stages of pregnancy (12) and rarely also in non-pregnant animals (5, 19). It was thought that in the non-pregnant cats in which this type of response was observed, the uterus had not completely returned to the resting state following a pregnancy, but no anatomical evidence for this assumption was found.

However the following observations (16 cats) show that, under suitable conditions, diphasic responses of the uterus can be obtained in every non-pregnant cat. During estrus, induced by injections of theelin, the movements of the uterus often, particularly in large animals, consist of powerful contractions separated by long intervals of rest. Stimulation of the hypogastric nerve, then, always elicited a strong contraction, followed by complete relaxation during which the muscle was unresponsive to further nerve stimulation (fig. 2A).

In smaller animals the uterus usually was in a state of tonic contraction on which only slight rhythmic contractions were superimposed. In this case stimulation of the hypogastric nerve always produced an initial contraction (fig. 2B).

The magnitude of the initial excitatory effect depends largely on body temperature. At normal temperature the effect sometimes could not be demonstrated clearly, but it was always observed, and markedly increased, after the body temperature was lowered to 33° to 36°. In some cases the

excitatory effect was the most prominent part of the response and inhibition followed only after prolonged stimulation.

The initial contraction occurred without any noticeable delay and direct observations showed that it began simultaneously with a blanching of the organ. This fact excludes the possibility that the contraction resulted from asphyxia due to vasoconstriction.

Diphasic responses to stimulation of the hypogastric nerve have been described also in other species. It was found by Cushny (4) in the rabbit,

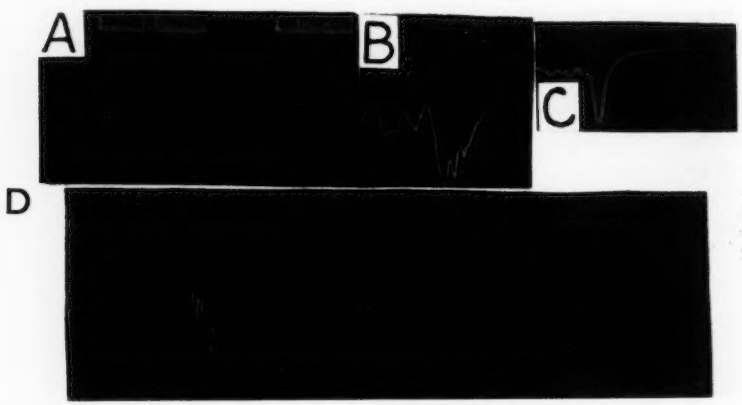

Fig. 2. Contractions of the cat's uterus in situ. The records show the initial contraction due to stimulation of the hypogastric nerve (A and B) and adrenaline (C and D).

A, uterus fully relaxed. The first upward movement of the lever is due to the contraction of the urinary bladder.

B, weak rhythmic contractions are increased by nerve stimulation (time intervals 6 sec.).

C, two injections of adrenaline (20 γ), time of injection indicated by signal.

D, responses to adrenaline (20 γ), time of injection indicated by upper signal. Time intervals in seconds. Because of the strong spontaneous contractions responses to single injections of adrenaline could not be demonstrated with certainty, but a second injection gave a contraction before a spontaneous contraction would have occurred.

and by Langley (13) in the dog (2 animals). These observations and the experiments just reported on the non-pregnant cat, where a purely inhibitory action of the sympathetic had been accepted, make it probable that diphasic responses occur more frequently than generally assumed. Therefore, contraction followed by inhibition may be considered as the general type of response of the uterus to sympathetic nerve impulses. The well known variations in the effects of nerve stimulation thus appear merely as quantitative differences in the magnitude of the excitatory and inhibitory phase of the response.

The diphasic responses are generally (cf. 10, 17) explained on the assumption that the hypogastric nerve contains excitatory and inhibitory nerve fibers. On the basis of the theory of chemical transmission of nerve impulses it appears logical to assume that these antagonistic nerve fibers are adrenergic and cholinergic (cf. 17). However atropine, even in concentrations many times greater than necessary for blocking the vagus, did not change the responses of the cat's uterus (in confirmation of Kennard's observations (12) on the pregnant uterus). Furthermore it is well known that in animals where sympathetic nerve impulses have an excitatory action also adrenaline increases motility. It is, therefore, probable that the excitatory as well as the inhibitory action of sympathetic nerve impulses is due to adrenergic fibers.

It is particularly significant that adrenaline alone, injected intravenously, can elicit diphasic responses. In every instance the action of this drug, whether it was purely inhibitory or diphasic, agreed closely with the effects produced by stimulating the hypogastric nerve (fig. 2). These facts do not exclude the possibility of a supply of cholinergic fibers to the uterus, but such fibers, if they exist, do not determine significantly the nature of the responses.

In agreement with the results of Kennard (12) on the pregnant uterus, the inhibitory phase often, but not always, was more prominent after injection of adrenaline than after nerve stimulation. This difference may be explained by the different rate at which the concentration of adrenaline rises in the muscle and does not indicate an essential difference in the responses. On the basis of the theory which will be presented below it may be expected that the initial contraction is the smaller the more rapidly the concentration of adrenaline rises within the musculature.

Diphasic responses produced by adrenaline have also been observed in the dog (20) and monkey (11).

To reconcile the fact that adrenergic nerve fibers, or even adrenaline alone, can produce in the same muscle excitation as well as inhibition, with the theory of specific excitatory and inhibitory nerve fibers, it becomes necessary to suppose that the antagonistic effects occur at different regions of the muscle. Kennard assumed that the excitatory and inhibitory action is due to the formation of two different kinds of sympathin liberated in different regions of the muscle. However this hypothesis disagrees with the fact that on continuous stimulation the initial contraction is followed by complete inhibition and that the muscle remains unresponsive thereafter for one minute or longer (fig. 2).

In view of the apparent impossibility of separating the excitatory from the inhibitory effects of sympathetic nerve impulses it seems worth while to consider the possibility that these effects represent two phases of the action of the same nerve impulses.

As stated above, inhibition may conceivably be brought about by one

or both of two changes: 1, interference with the processes responsible for the initiation of the impulses, like the depression of the pacemaker of the heart; 2, a decrease in excitability resulting in a block for muscular conduction, as demonstrated for cardiac and visceral smooth muscle.

Experimental evidence indicates that these two changes are essentially independent from one another. In the heart it has been shown that the initiation of beats is not determined by the level of electric excitability of the pacemaker (6) and, consequently, the slowing of the beat produced by vagal impulses cannot be considered as an effect secondary to the decrease in excitability. Furthermore it has been found that acetylcholine, although lowering the excitability of cardiac muscle (cf. 8) has only an inotropic and no chronotropic action on the pacemaker if applied in low concentration (7), showing that these two changes are separate effects of vagal stimulation.

Similar evidence was obtained for intestinal muscle. Adrenaline, although it decreases the excitability of the muscle, never diminishes the

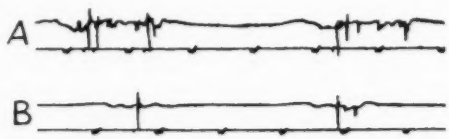

Fig. 3. Action potentials from the small intestine of the rabbit in situ. A, normal pendular movements: B, after intravenous injection of 15 γ adrenaline.

frequency of the movements, provided that the concentration of the drug is not high enough to stop the movements entirely.

Adrenaline diminishes the number of impulses discharged during each contraction (fig. 3). This effect is responsible for the decrease in the amplitude of the movements. It can be explained by the lowering of the average level of excitability, which cuts short each burst of impulses sooner than would normally occur.

The diphasic responses can be explained as follows. On the basis of the observations just reported it appears reasonable to assume that sympathetic impulses tend to initiate muscular impulses and that, at the same time, they lower excitability. It would be expected, then, that nerve impulses or adrenaline first increase activity. During continuous stimulation, however, the discharge of the muscle will be stopped by a block of muscular conduction as soon as the excitability has dropped below a certain level. Thus a diphasic response will result. If, on the other hand, the block is established rapidly the excitatory phase may be masked. Finally a purely excitatory action will be found if the excitability is not appreciably depressed by the sympathetic nerve impulses, as has been found under certain conditions (p. 629).

This theory explains the diversity of the responses of the uterus as the result of quantitative differences in the functional condition of the muscles. A reversal in the action of sympathetic impulses and adrenaline in some species, then, may be considered as an expression of the change in the functional state of the uterine musculature as it occurs during pregnancy and estrus, and it becomes unnecessary to postulate alterations in the function of the nerve fibers.

In conclusion it may be said that the hypothesis of specific motor and inhibitory sympathetic nerve fibers to the uterus has not contributed to the understanding of the diversified responses of this organ. This hypothesis seems on a rather insecure basis because purely excitatory and inhibitory effects of nerve stimulation are rather the exception than the rule, and it cannot account for the fact that excitatory as well as inhibitory effects are produced in the same organ by adrenaline alone or by adrenergic nerve fibers. It appears, therefore, that the assumption of a dual action of sympathetic nerve impulses offers a simpler explanation of the responses of the uterus than the hypothesis of a dual sympathetic innervation.

Also in other visceral organs it has seemed necessary to postulate the presence of excitatory and inhibitory sympathetic nerve fibers. In the urinary bladder (cf. 10, 15), adrenaline and stimulation of the hypogastric nerve produce a diphasic response like that of the uterus. In the gastrointestinal tract the sympathetic is predominantly inhibitory but excitatory effects are often obtained, particularly in the stomach (cf. 14). The conclusions regarding the nervous control of uterine motility may possibly be applied also to these observations, particularly to cases where adrenaline alone has a diphasic effect.

Several older investigators (cf. 10) reported an increase in the motility of the small intestine as a response to splanchnic stimulation. These observations were confirmed in experiments on 4 cats and 2 dogs. Contractions could be induced in the quiescent intestine by splanchnic stimulation, but these responses occurred only at very low body temperatures (33° to 30°) and they always had a long latent period (15 to 20 sec.). It seemed possible, therefore, that the contractions resulted from the asphyxia produced by vasoconstriction and, in fact, asphyxia produced by occluding the aorta also increased motility in these animals. It seems probable, therefore, that the sympathetic has a purely inhibitory action on the musculature of the small intestine.

SUMMARY

Sympathetic nerve impulses and adrenaline lower or abolish the excitability of the non-pregnant cat's uterus. Adrenaline was shown to produce this effect also on intestinal strips.

In the non-pregnant cat's uterus the inhibition brought about by stimu-

lating the hypogastric nerve is always preceded by an excitatory effect. This finding and occasional observations on other species suggest that diphasic responses are the general type of response of the uterus to nerve stimulation. The magnitude of each one of the two phases of the response varies greatly under different conditions and in different species.

Both the excitatory and inhibitory effects are produced by adrenergic nerve fibers as shown by the fact that adrenaline alone gives essentially the same response as sympathetic nerve impulses.

These results cannot readily be explained on the assumption of specific excitatory and inhibitory nerve fibers. It is suggested that excitation and inhibition represent two phases of the action of the same nerve impulses. Specifically, the observations are explained by assuming that inhibition is due to the diminution in excitability, which leads to a block of muscular conduction, and by the further assumption that the sympathetic nerve impulses also tend to set up muscular impulses. The excitatory action will be suppressed as soon as the level of excitability has dropped sufficiently to block muscular conduction. A diphasic response will result usually but one of the phases may also be masked by the other. These assumptions explain the great variety of responses of the uterus and the reversal of the responses during pregnancy or estrus, as the result of the experimentally demonstrated quantitative differences in the properties of the muscles without assuming any changes in the nervous mechanism.

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THE MEASUREMENT OF VENOUS PRESSURE IN MAN ELIMINATING THE HYDROSTATIC FACTOR

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The pressure at any point in a vein may be considered to be the result of the following factors: the residuum of the total head of pressure created by the heart beat which is transmitted through the capillaries to the vein, the resistance to the flow of blood from the point in the vein where the pressure is measured to the right auricle, the pressure in the right auricle, and the hydrostatic pressure exerted by the column of blood extending from the point in the vein where the pressure is measured to the point in the right auricle where the blood leaves the right auricle.

Most workers, in order to eliminate the hydrostatic factor, have taken the level of the right auricle as the point to which venous pressure should be referred. However, Clark, Hooker, and Weed (1934) have shown that in the dog the reference point, as they use it, is not the right auricle but consists of two points, one located several centimeters cephalad to the heart for the head section of the animal, and another located several centimeters caudad to the heart for the tail section of the animal. However, since blood leaves the venous system at the right auricle, and so long as there is a column of blood extending from the right auricle to the point in the vein where venous pressure is measured, there is a hydrostatic pressure equal to the height of this column of blood. In order to eliminate this hydrostatic pressure from the venous pressure measurement, it is necessary to refer the venous pressure to the level of the right auricle.

There is little agreement as to the position of the right auricle in the chest. The position of the right auricle in the supine subject has been taken to be at the following points by different workers: one-half the distance through the chest in the anterior-posterior line drawn from the subcostal angle (von Recklinghausen, 1906); five centimeters dorsal to the sternum at the fourth costal cartilage (Moritz and Tabora, 1910); at the junction of the anterior third and the middle third of the anterior-posterior line drawn from the sternum at the fourth intercostal space (Eyster, 1929); and ten centimeters ventral to the level of the skin of the back (Lyons, Kennedy and Burwell, 1938). These reference points differ widely and

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in some cases the extreme points differ by as much as ten centimeters; in most cases they differ by about four centimeters. This causes the venous pressure, as measured, to differ by this much.

It has been attempted here to determine more accurately the position of the reference point and to measure the venous pressure by a procedure that eliminates the hydrostatic factor in the measurement. If the venous pressure in the antecubital vein of a subject in the supine position is measured by the direct method, this pressure is composed of the hydrostatic pressure exerted by the column of blood extending from the point in the vein where the pressure is measured to the right auricle plus the remaining venous pressure which has no hydrostatic component (fig. 1a). If the subject is turned to the prone position and the pressure measured in a similar manner, this pressure is composed of a hydrostatic pressure

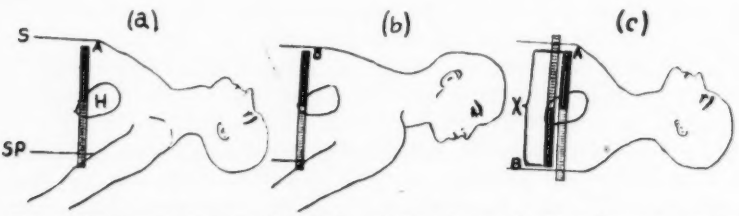

Fig. 1. Diagrams showing the height of the column of saline (venous pressure) in the supine and prone positions. Solid black area represents the venous pressure having no hydrostatic component. Shaded area represents the hydrostatic component. (a), supine. (b), prone. (c), composite picture of the two positions. *A*, level of the top of the saline column with the subject in the supine position. *B*, level of the top of the saline column with the subject in the prone position. *H*, heart. *S*, sternum. *SP*, spine. *X*, the sum of the two venous pressures with the hydrostatic pressure eliminated.

component equal to the height of the column of blood extending from the point in the vein where the pressure is measured to the right auricle plus the remaining pressure which has no hydrostatic component (fig. 1b). In the above two pressure measurements the position of the right auricle is not known, but the pressures can be referred to the level of the skin lying over the spine, at the level of the fourth intercostal space, which is a fixed point. If a composite picture (fig. 1c) is made of the two above measurements, and it is assumed that the position of the right auricle relative to the spine does not change when the subject changes from the supine to the prone position, then it is seen that the distance between the points *A* and *B* (fig. 1c) represents the sum of two venous pressure measurements, and that the hydrostatic pressures have been eliminated from the venous pressure measurement. If the venous pressure (less the hydrostatic component) does not change as a result of the change from the supine

to the prone position, then the mid-point between A and B (fig. 1c) represents the reference point in the right auricle.

Thus, if the venous pressure is measured with the subject in the supine position and then measured with the subject in the prone position, using the spine as the reference (zero) point in both cases, the sum of these two venous pressure measurements divided by two equals the venous pressure with the hydrostatic component eliminated; and the reference point is located at the point in the chest midway between the tops of the two columns of saline in the two pressure measurements, as shown in figure 1c.

It should be noted in determining venous pressure by this method that in the supine position when the top of the column of saline is ventral to the spine the pressure in the vein, using the spine as the reference (zero) point, is positive. When the subject is in the prone position and the top of the column of saline is ventral to the spine, the pressure in the vein, using the spine as the reference (zero) point, is negative.

Method. Venous pressure was measured in the antecubital vein in ten normal subjects in the supine position by a modification of the direct method of Moritz and Tabora (1910) using physiological saline in place of citrate in the manometer system. The subject lay on a flat table with a meter stick of 0.8 cm. thickness lying between the spine and the table in order to insure accurate location of the position of the spine. The thickness of the chest in the anterior-posterior diameter at the fourth intercostal space was measured. After the subject had been relaxed on the table for between ten and fifteen minutes with the extended arm lying on a smooth board posterior to the level of the spine and abducted to approximately forty-five degrees, the needle was inserted, taped in place and a venous pressure reading taken referring the pressure to the level of the spine as zero. The arm was raised a few centimeters by elevating the board on which the arm rested and then another pressure reading was taken. This was continued until the point in the vein where the pressure was measured was at the level of the sternum or above. The subject was then turned to the prone position, the needle remaining in place, and the arm extended, abducted to about forty-five degrees, and placed on a smooth board well below the sternum. The venous pressure was determined and then the arm elevated a few centimeters and another pressure reading was taken. This was continued until the point in the vein where the pressure was measured was approximately at the level of the spine. The level of the spine was determined and all pressure measurements referred to the spine as zero. In this way the venous pressure was determined in each subject using all of the reference points described above.

Results. With the subject in the supine position venous pressure was found to vary widely in each subject depending on the reference point used (table 1). Lower values for venous pressure were obtained using the refer-

ence point of Moritz and Tabora and that of Eyster than were obtained using the other reference points. Venous pressure values obtained using the reference point of von Recklinghausen were close to the values obtained using the method described here. The venous pressure values obtained using the reference point of Lyons et al. were close to the values obtained using the method described here in all cases except that of a very thick chested individual in which case the value obtained using the reference point of Lyons et al. was higher than that obtained by any other method.

TABLE 1

S	CHEST DIAMETER	VENOUS PRESSURE MEASURED IN CENTIMETERS OF SALINE									R. P.
		M. and T.		E.		v. R.		L. et al.		X	
		Supine	Prone	Supine	Prone	Supine	Prone	Supine	Prone	Supine and prone	
	cm.										
1	19.4	8.5	19.8	10.0	18.3	13.2	15.1	12.9	15.4	14.1	8.7
2	18.3	6.7	16.2	7.8	15.1	10.9	12.0	10.0	12.9	11.4	8.5
3	19.5	7.1	17.1	8.6	15.6	11.9	13.7	11.6	12.6	12.1	9.5
4	20.0	6.0	10.8	6.7	9.8	9.9	8.0	9.9	5.8	7.8	12.0
5	25.7	4.6	17.4	8.1	14.4	12.5	11.7	15.3	10.8	11.2	14.0
6	18.5	5.3	16.3	5.8	15.3	8.9	13.2	8.1	10.6	9.3	8.8
7	19.8	8.0	12.3	8.0	10.7	8.5	8.9	8.3	9.0	7.9	10.4
8	17.8	6.0	12.4	6.1	11.5	8.0	9.1	6.9	10.2	8.3	8.7
9	18.4	5.3	11.6	6.4	10.5	9.5	7.4	8.7	8.2	8.4	10.2
10	18.9	8.9	19.1	10.2	17.8	13.4	14.6	12.8	15.2	14.0	8.8
Av.....	19.6	6.6	15.3	8.0	13.9	10.7	11.4	10.4	11.1	10.4	10.0

Summary of data on venous pressure determinations in the supine and prone positions using different reference points. S, subject; M. and T., reference point of Moritz and Tabora; E., reference point of Eyster; v. R., reference point of von Recklinghausen; L. et al., reference point of Lyons et al.; X, using the method described here; R. P., reference point, as determined by the method described here, expressed in centimeters ventral to the spine; Av., average.

Although the reference points of von Recklinghausen, Moritz and Tabora, Eyster, and of Lyons et al. were not intended to be used in the determination of venous pressure with the subject in the prone position, the venous pressure in the prone position was measured using these reference points in order to show the wide variation of venous pressure depending on the reference point used (table 1).

The venous pressure measured with the subject in the prone position using the reference point of Moritz and Tabora and that of Eyster was high compared to the values obtained by using the other methods, while the opposite was the case with the subject in the supine position (table 1).

The normal venous pressure was found to vary between 7.8 and 14.1 cm. of saline with 80 per cent of the cases having a pressure between 7.8 and 12.1 cm. of saline using the method described here.

Using the spine as the reference (zero) point for the venous pressure measurement, it was found that raising or lowering the vein caused no change in the venous pressure so long as the vein remained below the middle region of the chest. Elevation of the vein above the middle region of the chest caused an elevation in the venous pressure (fig. 2). This was seen both in the supine and prone positions, and similar results were obtained on models using thin walled collapsible rubber tubes to represent veins.

Discussion. There is a possibility that in determining venous pressure by the method described here the shift from the supine to the prone position changes the venous pressure and the position of the right auricle, and thus the venous pressure, as measured, is inaccurate and the location of the reference point is inaccurate. That this is not the case is suggested by the following observations. Hooker (1914), White (1924), Doupe et al. (1938), and others have shown that vasodilatation or vasoconstriction in the arm causes little or no change in venous pressure, thus it is unlikely that the shift from the supine to the prone position causes any change in the pressure transmitted through the capillaries to the vein. Also, so long as the subject is relaxed and there is no constriction between the point where the venous pressure is measured and the heart, the resistance to flow to the heart is probably not changed by the change from the supine to the prone position. There is the possibility, however, that the pressure in the right auricle is changed as a result of the change from the supine to the prone position. Although there are no data on this point in man, intra-auricular pressure measurements in three anesthetized dogs, with the chests closed, showed that the pressure either did not change or was decreased by one centimeter of saline pressure when the animal was turned from the supine to the prone position. This suggests that there is little or no change in right auricular pressure in man due to the change from the supine to the prone position. Since the base of the heart is firmly fixed posteriorly, it is unlikely that the shift from the supine to the prone position causes any marked change in the position of the right auricle relative to the spine. However, if the position of the right auricle is changed due to the shift from the supine to the prone position, the reference point, as determined by the method described here, will lie at a point midway between the two positions of the right auricle (fig. 1c). Also, the venous pressure will be decreased, assuming that the heart falls forward in the prone position, by an amount equal to the pressure exerted by one-half the height of the column of blood extending between the two positions of the right auricle.

Thus it seems unlikely that the shift from the supine to the prone posi-

tion causes any change in the venous pressure. However, if the venous pressure does change due to the shift from the supine to the prone position then the value obtained for venous pressure using the method described here will be the average value of the venous pressure in the two positions with the hydrostatic factor eliminated.

The rise in venous pressure that was found when the arm was placed above the heart level confirms the observations of Carrier and Rehberg (1923) and those of Lyons et al. (1938). These investigators attributed this rise to the elevation of the vessels above heart level causing the

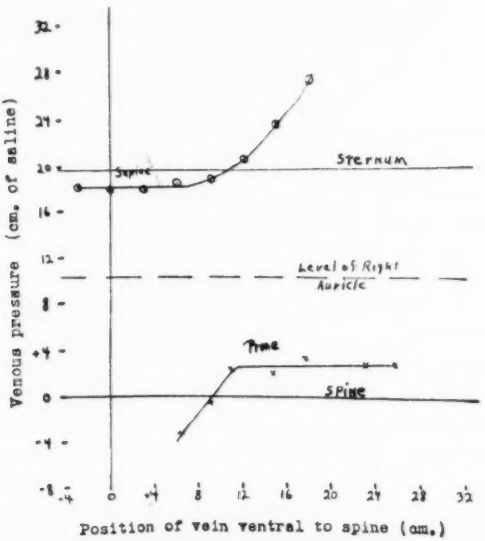

Fig. 2. Graph showing the height of the top of the column of saline (venous pressure) ventral to the spine with the arm in different positions relative to the spine. +, ventral to spine. −, dorsal to spine. The level of the right auricle was determined as described in the text.

vessels to collapse and thereby increasing the resistance to flow between the point where the pressure was measured and the heart, and thus increasing the venous pressure. However, there is the possibility that this rise in venous pressure is the result of constriction of the vein caused by angulation at the shoulder when the vein was above heart level. That this is probably not the case is shown by the fact that a rise in venous pressure was found with the subject in the prone position only when the vein was above heart level (fig. 2). Since angulation of the arm at the shoulder was the same with the vein above heart level in the prone position as it was with the vein below the heart level in the supine position

and since the rise in venous pressure was seen only when the vein was above the heart level, the angulation must have played no part in the pressure rise.

SUMMARY

Venous pressure was determined in the supine and prone positions in ten normal subjects using a modification of the direct method of Moritz and Tabora. Venous pressure values obtained using the reference point of Moritz and Tabora and that of Eyster were markedly different from the values obtained using the reference points of von Recklinghausen, Lyons et al., and the method described here. The reference point of Moritz and Tabora and that of Eyster appear to be placed too far ventrally.

It is suggested that venous pressure be determined in the following manner in order to eliminate the hydrostatic factor. The pressure in the antecubital vein is measured by the direct method with the subject in the supine position, the arm lying well below the center of the body and abducted to approximately 45 degrees. The subject is turned over into the prone position and the pressure measured again with the arm well below the center of the body and abducted to about 45 degrees. All pressures are referred to the level of the spine as zero. The sum of the two pressures divided by two equals the venous pressure, and the reference point is located at the point in the chest mid-way between the tops of the two columns of saline in the two pressure measurements as shown in figure 2.

Venous pressure determined in this manner varied between 7.8 and 14.1 cm. of saline with 80 per cent of the cases varying between 7.8 and 12.1 cm. of saline.

The author wishes to express his thanks to Dr. A. J. Carlson for his suggestions and criticism during the course of this work.

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RECIPROCAL INNERVATION IN THE SMALL INTESTINE

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The small intestine is supplied by two efferent nerves which affect its motility—the vagi and the splanchnics. Most investigators are in general agreement that the former are motor, the latter inhibitory (for a review of the evidence on this point see Hukuhara, 1932). The fact that an effector is supplied by two nerves, which on peripheral excitation produce opposite actions, does not necessarily mean that the organ is reciprocally innervated. It does, however, immediately suggest the possibility that this type of coördination is present. The experiments here described were designed to test whether reciprocal innervation does indeed exist in the gut. The method consisted of destroying either the motor or inhibi tory nerves and observing whether augmentor or depressant reflexes are still obtainable. If, when humoral agencies are excluded augmentor responses can be elicited in the absence of the motor nerves, or if inhibition is apparent after destruction of the depressant fibers, then—since only two extrinsic nervous sources are known to act on the gut—the altered motility must be mediated by a diminished activity of the remaining nerves. The persistence of such reflexes, then, would prove reciprocal control of an autonomic effector.

METHODS. Most of the experiments were performed on young vigorous cats, but in a few cases rabbits were used as test animals. In order to insure the presence of lively intestinal movements the following expedients were employed. 1. Sometimes a dose of castor oil was administered 14 to 24 hours before the experiment. 2. In all cases (regardless of whether or not a purgative had been given the day before) the animals were fed a meal of salmon and milk 2 to 3 hours before the start of the experiment.

The cats were anesthetized with urethane, 1.25 grams per kgm. in the femoral vein, or dial (Ciba) 0.65 to 0.75 cc. per kgm. intraperitoneally (slightly smaller doses were employed for rabbits). The animal was then placed on a warming pad and its temperature was kept within the normal range.

After the insertion of a tracheal cannula the abdominal cavity was opened. The influence of the adrenals was routinely abolished by tying off each gland *en masse* with stout thread. When the experiment required

removal of the splanchnics, these nerves were identified as they emerged beneath the diaphragm on either side and 1 to 2 cm. were extirpated. In some instances the semilunar ganglion also was cut out. A small rubber balloon, made of condom rubber, or more usually fashioned from the finger of an ordinary surgical glove, was attached to a glass tube of narrow bore by means of rubber cement. It was passed into the lumen of the duodenum through a short longitudinal incision. A stitch carried through both layers of muscle and tied around the glass tube held the balloon in place. The balloon was connected by rubber tubing and a T-tube to a Marey capsule and a water manometer. The pressure in the system was raised to 5 to 10 cm. of water and the manometer was then disconnected by means of a stopcock. Thus a record of duodenal contractions and the tone of the musculature was obtained on a kymograph. The system was tested for leaks before each experiment. In order to obviate the disturbing influences of drying, cooling and handling, the contents of the peritoneal cavity were carefully wrapped in a towel soaked in warm Ringer's solution, while the above procedures were being carried out. The surgery was performed as rapidly as possible and with care to prevent unnecessary trauma. After the operations were completed, a small quantity of warm Ringer's was introduced into the abdominal cavity, the guts were carefully arranged to avoid kinking and the omentum was spread over them. Then the abdominal wall was closed with hemostats or large paper-clips, or sewn with linen thread.

The sciatic nerve on one side was bared, cut, and prepared for central stimulation. The vagi on both sides were isolated and made ready for section or central excitation. In all experiments the cervical sympathetic trunks were separated from the vagi and cut. Generally, if the vagi were to be excluded, they were cut before any records were taken. In some cases, however, it was thought worth while to investigate a possible tonic action of these nerves. Hence, they were not cut until the experiment was in progress and the intestinal movements were being recorded.

In a few of the earlier animals the guts were allowed to remain within the closed abdominal cavity while the experiments were being conducted. It was frequently found, however, that there was a tendency for the motility to become seriously impaired after 1 to 2 hours. Consequently, the later experiments were carried out with the intestines floating in a Ringer bath, the temperature of which was kept constant at 38°C. This method had the advantage of preserving the vigor of the movements for several hours. It also allowed the advantage of direct observation of the gut and avoided the complications which contractions of the abdominal musculature occasionally created.

Some animals were chronically sympathectomized or vagotomized 7 to 16 days before the acute experiment. Bilateral abdominal sympathec-

tomy was performed aseptically according to the technique of Cannon, Newton, Bright, Menkin and Moore (1929). In addition, 1 to 2 cm. of the splanchnics were removed on both sides and the celiac ganglion was extirpated. Parasympathetic fibers to the small intestine were destroyed by stripping the vagal filaments from the anterior and posterior surface of the esophagus immediately after it pierced the diaphragm.

Shielded silver-wire electrodes were used for stimulation of the nerves. A Harvard inductorium with 1.5 to 3.0 volts in the primary circuit and varying positions of the secondary coil provided faradic induction shocks.

RESULTS. A. *Tonic action of the vagi.* Most investigators have concluded that the vagi are either entirely or primarily motor for the small intestine. This conclusion led to a study of the effects of section of these nerves to learn whether or not they exhibited a tonic action on intestinal motility. Bayliss and Starling (1899) were unable to detect, in anesthetized dogs, the existence of tonic vagal influence on the intestines. Hotz (1909) and L. R. Müller (1931) subscribed to the view of the English workers. The data of Aehle (1936) on a small series of rabbits are equivocal. Cannon (1906), in contrast to the foregoing investigators, assigned to the vagi a tonic action. He found, by means of x-ray studies carried out several days after cats had recovered from double vagotomy, that the passage of a meal of lean beef through the intestine was slower than normal. Also Alvarez and Mahoney (1924) stated that vagal section in rabbits reduced the tendency for "peristaltic rush" along the bowel.

In an attempt to add more data on the existence or non-existence of vagal tone, 8 cats were studied. After the intestinal movements were being recorded the vagi were cut in the neck. In 4 cats of this series the splanchnics had been previously sectioned, while in the remaining animals they were intact. In 5 experiments vagotomy resulted in a decrease in the rate and extent of the contractions. In 2 cases the results were not striking, and only a slight slowing of the movements occurred. In the other cat vagotomy was without effect on intestinal activity.

No statement can be made regarding the period of time during which effects referable to vagus section remained in the 7 animals. In some cases slowing or even complete inhibition appeared rapidly, and after a few minutes the rate approached the normal. The new rate, however, was always slower than before severing the vagi. Generally, the amplitude of contractions was first reduced and then gradually approached the normal in size. Figure 1 illustrates the most striking example of vagal tone. In the animal from which this record was obtained the splanchnics were cut, the adrenals were tied and the celiac ganglion was removed. Interruption of the vagal supply caused a prompt inhibition of all movements. Approximately 12 minutes later the intestines began to move rhythmically

again, although the contractions occurred less frequently and the individual beats developed less tension than before the vagi were cut.

B. *Reflex changes produced by sciatic nerve stimulation.* Excitation of an afferent nerve and various sensory stimuli, according to numerous observers, inhibit peristalsis and decrease intestinal tone. These observations were readily confirmed by faradizing the central stump of the sciatic nerve. Vagal section, either at the time of the experiment, or 1 to 2 weeks previously, did not abolish the reflex inhibition produced by sciatic stimulation.

Most investigators state that cutting the splanchnic nerves removes the possibility of obtaining reflex gastro-intestinal inhibition (Hotz, 1909; v. Lehmann, 1913; Morin and Vial, 1934). My results are in disagreement with this view. In the first experiments simple splanchnic section was performed and then the sciatic was stimulated. The presence of reflex inhibition led me to suspect—because of the contrary conclusions of earlier workers—that not all inhibitory fibers to the gut had been interrupted by splanchnicotomy; for it might be that a few fibers from the upper lumbar region ran, independently of the splanchnics, to the celiac ganglion. Consequently (in 8 cats) the celiac ganglion was removed when the splanchnics were cut. Figure 2 shows the typical inhibition after this possible source of error had been excluded.

In order to rule out any conceivable sympathetic supply and to obviate the possibility of humoral agencies (Youmans and Meek, 1937) 2 animals were subjected to bilateral abdominal sympathectomy and splanchnicotomy, removal of the celiac and superior mesenteric ganglia 9 and 16 days before the final acute experiment, at which time the adrenal glands were removed. These animals exhibited reflex intestinal inhibition, just as those in which less drastic surgery had been performed.

In the animals subjected to the different operations used to destroy possible sympathetic influences some variations in response occurred. Such were: latency, duration and extent of inhibition, and intensity and duration of the stimulus required to elicit the reflex. But the remarkable constancy of the inhibitory phenomena convinced me that they could be produced in the absence of all known depressant neural pathways. To prove that reflex inhibition was indeed mediated by the vagi—i.e., by a diminution in the normal vagal motor activity—these nerves were cut in the neck and the sciatic was again stimulated. Difficulties often arose in this phase of the experiment, for, frequently, when the vagi were destroyed the activity of the gut was permanently reduced (section A, p. 644) and the preparation was no longer so favorable for the study of inhibition. In some animals the gut remained fairly active after vagotomy. In such cases sciatic stimulation failed to evoke inhibition of the extrinsically denervated intestine.

Although a decrease in the rate and amplitude of the movements and a decline in the tone of the sympathectomized gut was the usual result of central sciatic stimulation, in 2 of 16 such experiments a marked *increase* in rhythm and tone occurred (fig. 3). The reason for these atypical results is obscure.

C. *Reflex changes induced by central vagal stimulation.* In view of the numerous reflex parasympathetic responses produced by central vagal stimulation this nerve was selected as one likely to produce augmentor intestinal effects. Conflicting reports concerning reflex vagal effects on the intestine can be discovered in the literature. Bunch (1897) at first stated that vagal stimulation was ineffective, but later (1899) considered

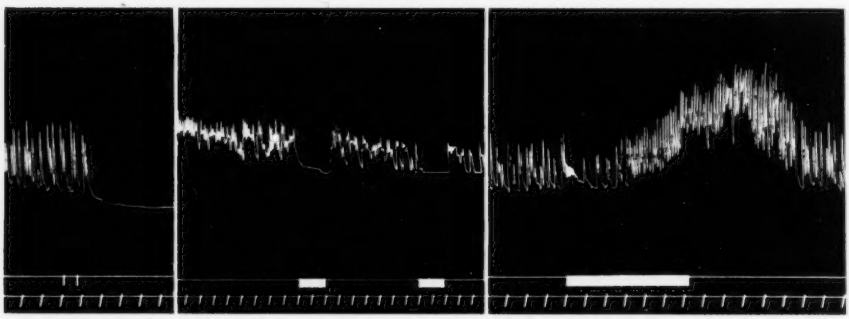

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Intestinal movements of cat under urethane. Splanchnic nerves cut, adrenals tied, celiac ganglion removed. Signals represent section of vagi in neck. In this and subsequent records the lowest line records 30-second intervals.

Fig. 2. Cat, urethane. Splanchnic nerves cut, adrenals tied, celiac ganglion removed. At signals, stimulation of central end of left sciatic nerve, 1.5 volts, 6 cm.

Fig. 3. Atypical record obtained on sciatic nerve stimulation. Cat, urethane. Splanchnic nerves cut, adrenals tied, celiac ganglion removed. At signal, right sciatic nerve stimulated centrally, 1.5 volts, 6 cm.

that it occasionally caused increased activity of the longitudinal coat if the other vagus was intact. Bayliss and Starling (1899) always observed inhibition if the vagus on the opposite side and the splanchnics were intact, and no effect if the splanchnics were cut. v. Lehmann (1913) secured either increases or decreases of peristalsis in the dog when the splanchnics and one vagus remained.

Under the conditions of my experiments, when the splanchnics were intact, increased motility was always brought about by stimulating one vagus centrally, provided the opposite nerve was untouched. Figure 4 is representative of 5 experiments, in each of which augmentation of motility followed faradization of the vagus. Even when the guts were

more active than in the animal from which figure 4 was obtained, vagal stimulation elicited a definite, though not so marked increase of contractions.

When one intact vagus represented the sole extrinsic nerve supply central stimulation of the other vagus also produced heightened motility. This response was abolished when the remaining vagus was severed.

If both vagi were sectioned and only the splanchnics connected the gut with the central nervous system, stimulation of the central end of either vagus brought about varying results. In 2 animals no alteration of intestinal activity occurred. In 2 others the intestines were inhibited. In the remaining 4 cats evidence of reciprocal innervation was obtained (fig. 5). That is, if distant humoral effects may be excluded, the increased movements could have been induced only by inhibition of the normally

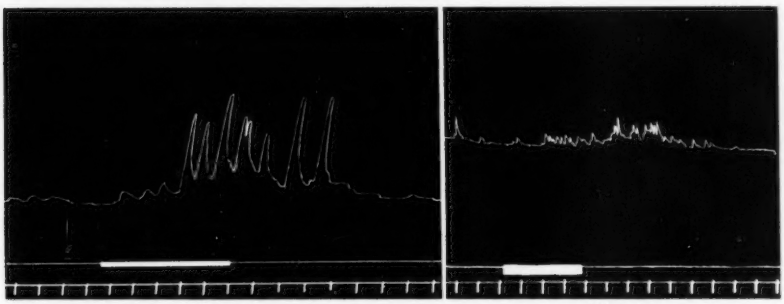

Fig. 4 Fig. 5

Fig. 4. Cat, urethane. Adrenals tied. Splanchnic nerves and one vagus intact. At signal, stimulation of central end of left vagus, 3 volts, 6 cm.

Fig. 5. Cat, urethane. Both vagi cut in neck. Splanchnics intact. At signal, right vagus stimulated centrally, 1.5 volts, 9 cm.

depressant influence of the splanchnics. The changes in motility were not due to chance variations in the intrinsic rhythmicity of the gut, since, after the splanchnics also were removed, neither inhibition nor augmentation took place when the vagus was stimulated. This operation, moreover, excluded the possibility of humoral depressant or excitant substances, released by nerves other than the splanchnics and acting upon the gut.

Discussion. The experiments reported in section A show that the vagi exert a tonic motor influence on the movements of the intestinal tract, since the effect of destroying these nerves is to reduce the rate and amplitude of the contractions (fig. 1). The decline in motility after double vagotomy might be assigned wholly to the now-unchecked reign of tonic and reflex inhibitory activity of the splanchnic nerves. The vagi act, however, not merely to offset the splanchnics but are required for establish-

ment of a tonic state, because peristaltic depression occurs even when the vagal section follows splanchnic section.

The results of reflexly induced changes of intestinal motility cannot be adequately accounted for on the basis of complicating circulatory changes. In the first place, the evidence of Van Braam-Houckgeest (1874) and of Bayliss and Starling (1899) and others proves the independence of motility from local vascular changes. Also, from the present experiments it is apparent that reflex inhibition may occur with either a pressor (sciatic) or depressor (vagal) response. The objection might be raised that both these circulatory changes could be responsible for local asphyxia—the rise in pressure because of the constriction of the splanchnic arterioles, and the pressure fall by pooling of blood in the viscera and stagnation resulting from the decreased blood flow. On that basis, however, one could not explain the occasional enhanced motility seen on sciatic stimulation (fig. 3), nor the motor effects following central vagal excitation after unilateral or bilateral vagotomy (figs. 4 and 5).

Intestinal inactivity arising from sciatic stimulation when the sympathetic nerves are destroyed is not due to an outpouring of adrenaline, since the adrenal glands were excluded routinely. Nor is it likely that the reflex release of sympathin can account for the changes, because the latencies are in most cases too short to allow action of a distant humoral agent (fig. 2). The precautions described above (p. 645) to insure complete sympathetic denervation render improbable the participation of an uncontrolled inhibitory neural agency. Furthermore, the inhibitory phenomena are abolished when the vagi are cut. These facts point to decrease of vagal activity as being the mechanism by which the reduced motility in the experiments under discussion was mediated.

Youmans and Meek (1937) state: "There is no indication that the vagal fibers play any part in gastro-intestinal inhibition thus produced" (by rectal stimulation). Most investigators have reached similar conclusions regarding inhibition of the bowel. The reasons for the divergence between my results and those of other workers is not clear, but a number of possible causes for the discrepant findings may be advanced. 1. Differences in anesthetics may account in part for the conflicting data. 2. Some workers employed drugs, e.g., atropine (Bayliss and Starling, 1899; Bunch, 1899) and curare (v. Lehmann, 1913) which may have prevented elicitation of the reflex. 3. Different types of afferent stimulation have been used by various experimenters, and hence the conditions were not the same. 4. Perhaps most important in considering the discordant findings is the experimental animal employed. The majority of studies have been carried out on dogs, and the species differences between the cat and dog may well afford a possible solution of the discrepancies.

The results of central vagal stimulation are not so conclusive as those occurring after sciatic nerve excitation. In contrast to v. Lehmann (1913),

I found that augmentor effects always followed when the other vagus was intact (fig. 4). It is possible that v. Lehmann's inhibitory effects might have been due to his use of stronger induction shocks than those employed in these experiments. He states that more intense stimuli are required to bring on inhibition than augmentation. Since the adrenals were not removed in his experiments, the possibility of humoral effects was not excluded.

The variable response elicited by central vagal stimulation when the splanchnics alone are present is puzzling. M'Crea, M'Swiney and Stopford (1925) state that the results of direct stimulation of the vagus depend on the condition of the gastro-intestinal musculature at the time of the stimulation. When the smooth muscle is in a state of high activity vagal excitation may cause inhibition, while augmentation results if the peripheral structures are in a condition of lowered activity. The possibility of a similar situation existing when intestinal reflexes are set up is not excluded, but the present experiments offer no indication as to the validity of this supposition.

The appearance, in 4 cases, of enhanced motility mediated by splanchnic depression (fig. 5) indicates the possibility of obtaining a reciprocal synergism between the motor and inhibitory systems, since effects mediated by agents other than the splanchnic nerves were ruled out (p. 647).

One further point may be mentioned regarding the relative ease of obtaining evidence of reciprocity for inhibition and the difficulty of securing similar proof for augmentation. In the normal animal conditions of stress may arise not infrequently and require an efficient and rapid mechanism for initiating a decrease in the activity of the alimentary tract; then both efferent systems are correlated to occasion the most economical production of the standstill of the gastro-intestinal canal. It should also be emphasized that humoral agencies (adrenaline and sympathin from distant sources), which begin their action after a longer latency and have more prolonged effects, undoubtedly coöperate with the neural mechanisms in keeping the activity of the bowel depressed when conditions which demand its quiescence arise. On the other hand, situations which require increased motility of the intestines may be accomplished in a more leisurely fashion and need not requisition the aid of both nervous pathways. A reciprocal action in the latter case is not precluded; indeed, the evidence is suggestive that it actually may take place. The implication is simply that in the latter situation reciprocity is not so highly developed as when the gut must be inhibited to meet the exigencies of normal existence.

SUMMARY

1. The movements of the small intestine were studied by the balloon method in cats and rabbits under urethane or dial.

2. The vagi exert a tonic motor influence on the small intestine (fig. 1; p. 644).

3. Faradization of the central stump of the sciatic nerve usually causes intestinal inhibition and a loss of tone. The decline in activity takes place even after the splanchnic nerves are destroyed (fig. 2). The diminished motility is shown to be due to a decrease in the normal activity of the vagi—hence, reciprocal innervation (p. 645).

4. In rare instances increase in movements and tone follows sciatic stimulation (fig. 3).

5. Central excitation of one vagus, with the other vagus and splanchnics intact, produces enhanced motility (fig. 4).

6. When both vagi are removed and the splanchnics are intact, central stimulation of one vagus causes either augmentor (fig. 5) or inhibitory reflexes. These are shown to be mediated by the splanchnic nerves (p. 647) and the augmentor reflex is advanced as possible evidence of reciprocal innervation (p. 649).

7. The significance of reciprocal innervation in the gastro-intestinal canal is briefly discussed (p. 649).

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DARK ADAPTATION AND EXPERIMENTAL HUMAN VITAMIN A DEFICIENCY[1]

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I. REASONS FOR THIS RESEARCH. The use of a visual function like dark adaptation to record the vitamin A state of the organism rests on two bodies of knowledge. The first is the evidence accumulated in the last 15 years which shows that vitamin A is the critical agent in the long-established association of night blindness with dietary irregularities. Night blindness in rats may be produced by a diet deficient in vitamin A, and this condition is cured by ingestion of vitamin A (Holm, 1925). Moreover, there is less visual purple formed in the eyes of A-deficient animals (Tansley, 1931), and it is regenerated more slowly during dark adaptation (Fridericia and Holm, 1925) than in normal animals. In human beings night blindness has been experimentally produced by an A-deficient diet (Jeghers, 1937; Hecht and Mandelbaum, 1938; Wald, Jeghers and Arminio, 1938) and the condition has also been reversed by resumption of a normal diet.

The second line of work is more recent, and has demonstrated that vitamin A is an essential ingredient of the chemical makeup of the visual system. Vitamin A is found in the retina (Wald, 1933), and appears as an ultimate product of the bleaching of visual purple (Wald, 1935). Thus vitamin A is a precursor for the formation of the visual pigments and is also a product of their decomposition,—a cycle which accounts for its necessity in vision, and which renders reasonable the employment of visual tests for the estimation of vitamin A in the body.

The visual test most commonly used for this purpose is some aspect of dark adaptation. However, in spite of its widespread clinical use in the diagnosis of human vitamin A deficiency, the precise behavior of dark adaptation during experimental vitamin A deficiency has not been established. This is first because only a few people have actually been studied experimentally, and second because even among these, differences have appeared in the experiments of different laboratories. The differences concern *a*, the time when a rise of the visual threshold first appears during the period of an A-deficient diet; *b*, the effect of single in-

[1] This work was aided by a grant from the Rockefeller Foundation.

gestions of vitamin A or of carotene on the visual threshold following a deficient diet, and *c*, the rate of recovery of the visual threshold after the resumption of a normal diet.

In order to supply material for the elucidation of these differences, we have made experiments in human vitamin A deficiency with 17 male subjects between 21 and 30 years old. Of these, 4 were studied during 1937–38 and have already been reported on briefly (Hecht and Mandelbaum, 1939); the other 13 were studied during 1938–39. The subjects were maintained on controlled diets for different periods and their dark adaptation was followed at frequent intervals.

II. Course of dark adaptation. The precise characteristics of dark adaptation depend on many factors such as the retinal location, the color and the size of the test area, as well as the duration and extent of the preceding light adaptation (Hecht, 1937). In order to maintain these factors constant, we used the apparatus devised by Hecht and Shlaer (1938) and the standard procedure recommended by them. This has the advantage not only of furnishing data whose characteristics possess physiological meaning, but of enabling the data to be related to the normal distribution of adaptation properties which we have already studied with a population of 110 individuals (Hecht and Mandelbaum, 1939). The procedure involves light adaptation to 1500 millilamberts for 3 minutes, and measurements during the subsequent darkness of the threshold of a retinal area 3° in diameter situated 7° nasally for flashes of violet light 0.2 second in duration.

For purposes of illustration, the course of dark adaptation of subject F. W. is given in figure 1. The lowest points and curve record the subject's performance during his period on a normal, balanced diet, and are typical of all the other subjects. As always, the course of adaptation shows two stages which are now well understood in terms of the double nature of human vision. The primary rapid drop in threshold is generally recognized as cone adaptation, while the secondary, slower and more extensive drop in threshold is the expression of rod dark adaptation.

The other measurements in figure 1 were made at different times during the subject's stay on a vitamin A-deficient diet. They show that the form of the adaptation curve remains essentially the same, but that the two parts of the curve become displaced upward in threshold. The cone threshold does not rise as much as the rod threshold, though their course is approximately parallel. This has already been found by us before (Hecht and Mandelbaum, 1938), and is confirmed by the work of Wald, Jeghers and Arminio (1938), of McDonald and Adler (1939), and of Wald and Steven (1939).

Because of this upward displacement of the adaptation curve as a whole, the best single criterion of visual sensibility under these conditions is the

final rod threshold, usually reached in about 30 minutes of darkness. In presenting the results of our dietary experiments we shall give only this final rod threshold.

It should be pointed out that though this is an adequate treatment for the present experiments, there are circumstances when such a single datum is an inadequate expression of the changes in dark adaptation. An example is the progress of dark adaptation during alcoholic liver cirrhosis (Haig, Hecht and Patek, 1938; Patek and Haig, 1939) when not only the final cone and rod thresholds change, but the two segments of the

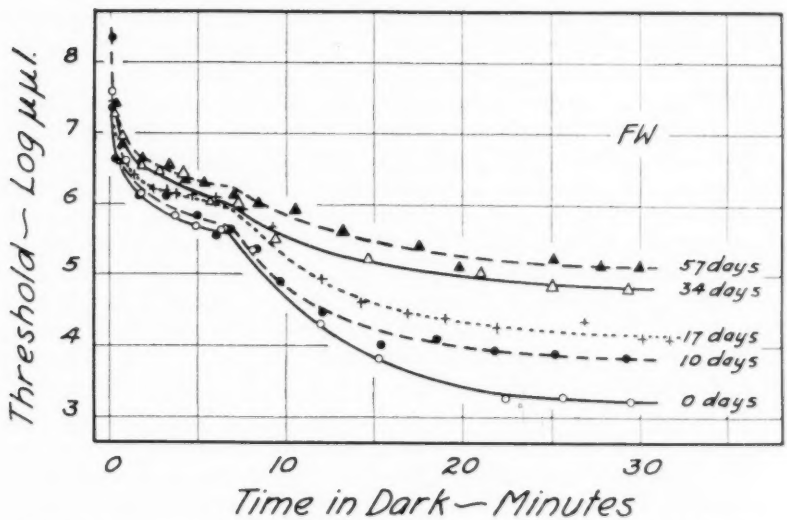

Fig. 1. The course of dark adaptation of a typical subject on a normal diet and at different times on an A-deficient diet. The ordinates are the threshold intensities given in log micromicrolamberts. The rod threshold at 30 minutes shows the largest effect of the diet and because it may be established at leisure, furnishes the best single criterion of the change during the diet.

curves shift relative to each other so that cone-rod transition time undergoes a steady change as well.

The curves in figure 1 show the futility of using a single point in the early stages of dark adaptation as a criterion of visual behavior. Clinical instruments like the biophotometer (Jeans and Zentmire, 1934; Jeghers, 1937) and the one described by Pett (1939) rely on rapidly made readings in the first stages of dark adaptation. Clearly, because of the great speed of adaptation at the beginning, measurements in this region are most difficult to make with precision, and are therefore least reliable. More-

over, the changes which the threshold undergoes during the A-deficient diet are least near the beginning of adaptation. It is hardly surprising therefore that such measurements are subject to statistical anomalies (Isaacs, Jung and Ivy, 1938) which frequently make them useless (Palmer and Blumberg, 1937). Conclusions drawn from results with instruments and procedures of this kind should be viewed with critical skepticism. It is to be hoped that whenever possible in clinical or dietary studies complete dark adaptation curves be made so that the precise visual effects may become known.

III. Vitamin A deficiency and visual threshold. The first difference which has arisen in the work of different investigators concerns the time of appearance of changes in visual sensitivity following the adoption of a vitamin A-deficient diet. With 4 subjects placed on a deficient diet we found an immediate and rapid rise of cone and rod threshold (Hecht and Mandelbaum, 1938). With their one subject, Wald, Jeghers and Arminio (1938) similarly reported an immediate rise in threshold, which has since been confirmed on an additional subject by Wald and Steven (1939). However, other investigators secured no such immediate rise, and some found none at all. Thus the 5 subjects used by Booher, Callison and Hewston (1939) required 16, 27, 29, 39 and 124 days respectively on a vitamin A-deficient diet before showing a recognizable rise in threshold. Moreover, Steffens, Bair and Sheard (1939) report only slight and temporary rises in threshold for 3 subjects on a deficient diet for about six months. In fact, Wald and Steven state briefly that some of their subjects failed to show any rise in threshold when placed on the same regime as the subject which responded sharply.[2]

From our own experiments we can say that the work of all these investigators is undoubtedly correct. There is an astonishing variability in the behavior of individuals, and our records can duplicate all the results previously reported.

a. Experimental procedure. The procedure used was essentially the same with all of our subjects. While they were on their normal diets we measured their dark adaptation at frequent intervals (daily or every other day) for about two weeks. After their normal threshold level was established, the subjects were put on a diet which was restricted to foods calculated from the tables of Daniel and Munsell (1937) to yield about 150 International Units of vitamin A daily. Their dark adaptation was measured frequently during the diet and the behavior of the threshold recorded in relation to the diet.

[2] Doctor Wald in a personal communication has recently amplified this. Of 4 additional subjects who have been kept on the deficient diet, 3 failed to show any rise in rod threshold after 2 months, and one subject has shown no threshold rise even after 4 months.

The 17 subjects on the deficient diet were treated in three groups as shown in table 1. The first group of 4 subjects received no supplements of other vitamins during the A-deficient dietary period. The second group of 11 subjects received as daily supplements 125 units each of vitamins B_1 and G in the form of brewer's yeast, and 1800 of vitamin D as irradiated ergosterol. In addition each subject had at least 300 cc. skimmed milk daily, and was encouraged to eat grapefruit for vitamin C. The third group of 2 subjects received daily supplements of 200 units of

TABLE 1

Effect of a vitamin A-deficient diet on the visual thresholds of 17 young men

GROUP	DAILY SUPPLEMENTS	SUBJECT	INITIAL THRESHOLD LOG $\mu\mu$l	DAYS ON DIET	TOTAL RISE IN THRESHOLD LOG $\mu\mu$l
I	None	H. M.	3.00	43	0.90
		J. M.	2.80	43	1.10
		J. Mi.	3.15	36	0.90
		L. W.	2.85	36	2.05
II	125 units B_1	J. B.	2.95	60	1.05
	125 units G	F. C.	3.10	47	0.55
	1800 units D	D. J.	3.10	124	1.60
	300 cc. skimmed milk	S. K.	2.90	103	1.05
		H. L.	2.65	59	1.00
		W. L.	2.75	94	2.00
		D. R.	2.65	43	1.15
		F. S.	2.95	42	0.70
		J. S.	3.30	109	1.70
		W. S.	3.15	103	1.00
		F. W.	3.15	82	2.15
III	200 units B_1	B. R.	3.15	37	1.00
	200 units G	H. S.	3.05	38	1.15
	2000 units D				
	50 mgm. C				
	300 cc. skimmed milk				

B_1 and G, 2000 units of D, and 50 mgm. of ascorbic acid, as well as at least 300 cc. of skimmed milk.

b. Results. Figure 2 shows the rod thresholds for each subject during the normal diet period and during the deficiency diet period. The subjects have been arranged in a rough order corresponding to the speed with which the threshold rose during the deficiency period. The beginning of this period is marked with a heavy vertical line.

Out of the 17 subjects, 14 responded at once to the diet with an unmistakable rise in threshold. The other 3 subjects need separate comment.

F. C.'s threshold rose so slowly over a period of 45 days that one can hardly be sure of it; his experimental diet was terminated for extraneous

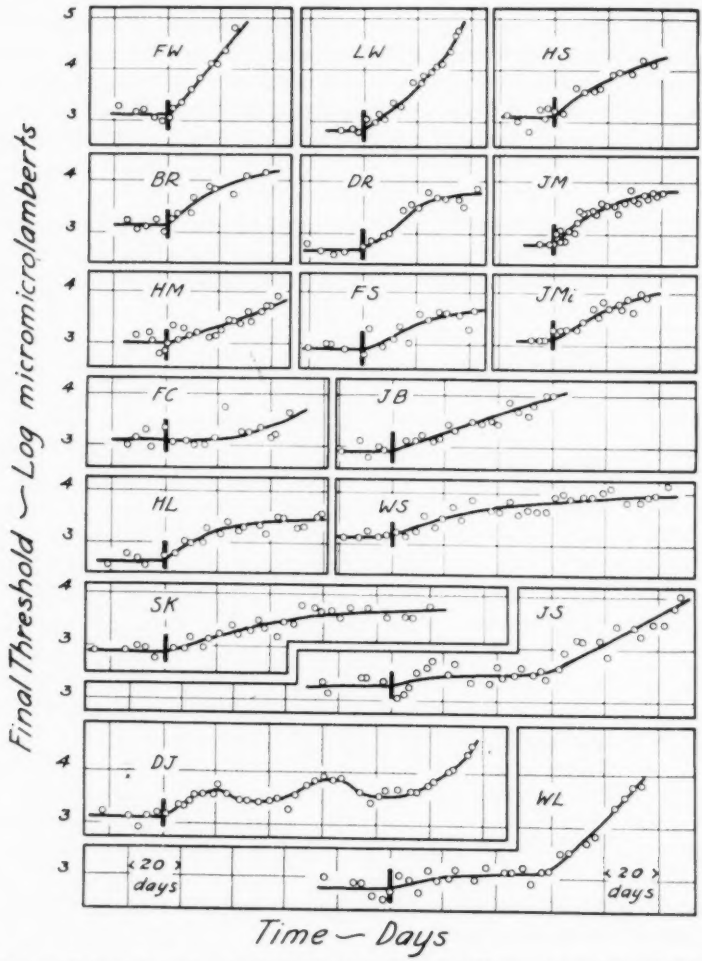

Fig. 2. Effect of a vitamin A-deficient diet on the 30 minute rod threshold of 17 young men. The black vertical line marks the beginning of the diet. In most cases the threshold begins to rise at once.

reasons. J. S.'s threshold hardly rose over a period of 60 days, and then suddenly began to rise sharply and unmistakably. An almost identical course was followed by W. L., who showed a very doubtful rise for about

60 days, and then suddenly began to rise in threshold with a speed comparable to the most spectacular behavior such as that of F. W. or L. W. One would hardly have blamed an experimenter for terminating the procedure with J. S. and W. L. after six or seven weeks and concluding that the visual threshold shows no change on a vitamin A-deficient diet.

Subject D. J. resembles in some ways those reported by Steffens, Bair and Sheard. On the deficient diet his threshold first rose unmistakably; but it soon stopped and even dropped, only to go through another cycle of up and down. By the end of 3 months, though the threshold was about 0.5 log unit above the initial normal, one would have hesitated to ascribe the rise to the diet, and one could have terminated the experiment as doubtful or even negative. However, at the end of the third month the threshold began to rise rapidly and extensively in the typical way already reported.

Supplementation of the A-deficient diet with other vitamins does not appear to influence the behavior of the threshold. Of the two subjects showing the most immediate responses, F. W. was in group II and received vitamin supplements, while L. W. received none. Similarly out of the next group of 7 subjects showing about the same degree of response, 3 were in group I, 2 in group II, and 2 in group III. Moreover, the 3 subjects who showed no immediate effects of the diet were all in group II which received adequate supplements of the other vitamins.

The rise in threshold is unrelated to the initial normal threshold. This is evident by simple inspection. However, we computed the coefficient of correlation between the initial threshold and the extent of the rise after 36 days on the deficient diet. The coefficient is -0.25 which is negligible. Similarly the correlation between the initial normal threshold and the average rate of threshold rise also yields an insignificant coefficient, -0.31.

The rate of the rise in threshold during an A-deficient diet may be taken as a measure of the vitamin A status of an individual. If this is so, then the lack of significant correlation between initial threshold and rate of rise during the deficient period indicates that within the normal range the threshold level itself can not be taken as an index of the nutritional state of an individual. This confirms our previous study (Hecht and Mandelbaum, 1939) of a university population of 110 individuals.

c. Subjective symptoms. In contrast to the reports by Jeghers (1937) and by Pett (1939), only two of our subjects recorded subjective awareness of their raised visual thresholds. The subjects are F. W. and L. W., and as figure 1 shows, they gave the most extreme and most immediate visual response to the deficient diet. At the very end of his deficient period L. W. reported an accidental collision with a wall at home due quite obviously to his raised visual threshold. Similarly F. W. near the end of

his deficient period observed that a longer interval of waiting in the fluoroscopy room was necessary before he could make observations.

None of our subjects reported any skin symptoms, and we observed none. There were no signs of epithelial keratinization of the eye, which confirms a similar lack of findings by Booher, Callison and Hewston, even on their subject whose threshold rose 3.5 log units on an A-deficient diet. In the light of our experience the report by Pett (1939) of anorexia, mild diarrhea, sore throat, rhinitis, decrease of salivary flow, gingival ulcers, dry, scaly skin, and painful eyes, all in one person on an experimental A-free diet must be considered as exceptional.

d. Conclusion. From our results it is fair to conclude that in most cases (14 out of 17) when a person is put on a vitamin A-deficient diet his visual threshold will rise almost immediately and will continue to rise so long as the diet is maintained.

The failure of some individuals to respond for as long as three months may be due to an exceptional storage of vitamin A in the tissues. If this is so, then the reserve can be drawn upon for long periods without signs of visual deficiency. When, however, a threshold change does begin to appear, the rate of change, and therefore the rate of storage depletion, is quite rapid.

Storage capacity for vitamin A apparently has no relation to the amounts recently ingested. Steininger, Roberts and Brenner (1939) found it to depend more on individual capacity and on long term history. This is borne out by the experience of Wald and Steven, who gave all their subjects a short period of high vitamin A dosage, and still got widely different individual effects with the subsequent deficient diet.

IV. Single doses of vitamin A. The second difference which has appeared in work from different laboratories concerns the effectiveness of single large doses of vitamin A in restoring the visual threshold after its rise on an A-deficient diet. There have been clinical reports of night blindness which has been cured within 24 hours by single oral administrations of a large quantity of vitamin A (*e.g.*, Aykroyd, 1930; Lewis and Haig, 1940), as well as by intramuscular injections of vitamin A concentrates (Edmund and Clemmesen, 1936) in even shorter time. In addition, Jeghers recorded the rapid recovery from experimental night blindness following the eating of large quantities of vitamin A. The most dramatic data are those of Wald, Jeghers and Arminio (1938), and of Wald and Steven (1939) obtained with a single subject each, in which the return of the visual threshold to normal occurs within minutes after ingestion of even moderate concentrations of vitamin A and of carotene.

Other investigators have not been able to secure similarly rapid recoveries. We (Hecht and Mandelbaum, 1939) obtained only slight effects with two subjects whose threshold had risen 1 and 2 log units above

normal as a result of a deficient diet, when they received oral doses of vitamin A concentrates containing 100,000 units. Similarly McDonald and Adler (1939) were unable to influence with single doses the visual threshold of their one subject which had risen on a deficient diet. Essentially the same findings are reported by Steffens, Bair and Sheard.

Our two subjects referred to above differed from those of Wald and his co-workers in having received no supplements of the other vitamins during their A-deficient period, and we were inclined to attribute the differences to this. We have now studied 7 additional subjects, all of whom had received supplements of other vitamins; 2 were in group III, and 5 in group II. The results do not bear out such an hypothesis.

a. Procedure. The experiments were made in two ways. A subject whose threshold was high as the result of an A-deficient diet had his dark adaptation measured in the usual way. When the threshold levelled off after 30 or 40 minutes, the subject, while still in the dark, was given a single dose of *oleum percomorphum* or of a concentrate containing a high value of vitamin A. During the next few hours the subject remained in the dark, and at frequent intervals his threshold was determined in order to see whether the ingested vitamin A produced any effects.

The first experiments with this method produced a good deal of boredom but little change in threshold in the course of several hours. The method was therefore abandoned for the following one. The subject had his dark adaptation measured in the usual way. He was then given a large dose of vitamin A and permitted to do what he wanted in the laboratory. Every hour or so after this, his dark adaptation was again measured with the standard procedure and the adaptation curves compared with the one secured before the single dose had been taken.

b. Results. The outcome was disappointing. Not one of our subjects, two of whom we tested on three separate occasions, responded with a complete return of the threshold to normal. However, most of our subjects, as the result of single large doses of vitamin A, showed some drop in threshold which varied in extent among the different individuals.

Figure 3 illustrates the course of some of the experiments. The solid circles connected by a vertical line represent the maximum change in threshold following single ingestions of about 100,000 units of vitamin A. The greatest effects were secured with D. J. and F. W., but in neither instance did the threshold return even half way to normal (on a log intensity scale).

The three successive trials with F. W. are significant in showing that on the day following each ingestion the threshold came back to its previous high position. Thus the effect of these single doses was temporary and cannot be considered as "cures" of night blindness.

Table 2 gives a summary of the findings with the 9 subjects. It is

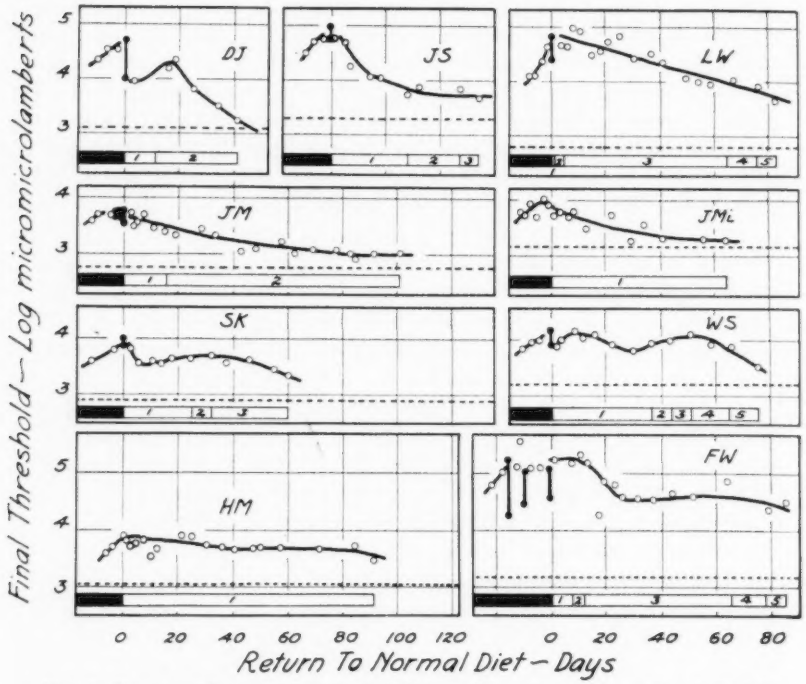

Fig. 3. Recovery from a vitamin A-deficient diet. In each case the dotted line represents the original threshold before the deficient diet. The black band marks the final part of the deficient diet, and the last few of the measurements of the deficient period are shown. The dark circles connected with heavy vertical lines record the effects of single ingestions of large doses of vitamin A. The clear band represents the return to a normal adequate diet. The numbers in this band indicate the following daily supplements:

D. J. (1) 25,000 units vitamin A; (2) 60,000 units A, 200 units B_1, 200 units G, 2 oranges.

J. S. (1) 25,000 units A; (2) no supplements; (3) 60,000 units A, 200 units B_1, 200 units G, 50 mgm. C.

L. W. (1) 50,000 units A; (2) no supplement—ill; (3) and (4) same as 1; (5) same as 1 plus 30 grams bile salts.

J. M. (1) 50,000 units A; (2) 50,000 units carotene.

J. Mi. (1) no supplements.

S. K. (1) 25,000 units A; (2) 60,000 units A; (3) same as 2 plus 200 units B_1, 200 units G, 2 oranges.

W. S. (1) 25,000 units A; (2) same as 1 plus 120 units B_1 and G; (3) same as 1 plus 200 units B_1 and G; (4) 50,000 units A, 200 units B_1 and G; (5) 120,000 units A, 200 units B_1 and G.

H. M. (1) no supplements.

F. W. (1) 25,000 units A; (2) no supplements; (3) same as 1; (4) 40,000 units A, 200 units B_1 and G; (5) 100,000 units A, 200 units B_1 and G.

apparent that the responses to single doses are as variable as were those to the deficient diet. The largest decrease in threshold is 1.00 log unit; the smallest is 0.05 log unit, which is really no effect, since a change of threshold of less than 0.15 log unit cannot be considered as reliable. The extent of the temporary threshold recovery does not seem correlated with the time during which the subject had been on the deficient diet, nor with the size of the threshold rise produced by the deficient diet, nor indeed with the supplementary vitamins given during the diet.

In one case (D. J.) we gave not only a high concentration of vitamin A but bile salts to aid in emulsification and absorption. The subsequent

TABLE 2

Effect of single large doses of vitamin A on the visual threshold of subjects on a vitamin A-deficient diet

GROUP	SUBJECT	DAYS ON DEFICIENT DIET	TOTAL RISE OF THRESHOLD ABOVE NORMAL LOG μl	UNITS OF VITAMIN A IN SINGLE DOSE	MAXIMUM DROP IN THRESHOLD LOG μl	TIME FOR APPEARANCE OF MAXIMUM DROP
						hours
I	J. M.	41	1.10	11,000	0.05	8
		42	1.10	30,000	0.10	8
		43	1.10	50,000	0.20	8
	L. W.	35	2.00	100,000	0.40	6
II	D. J.	124	1.60	100,000*	0.70	3¼
	S. K.	103	1.05	100,000	0.11	5
	J. S.	109	1.70	100,000	0.23	3½
	W. S.	103	1.00	100,000	0.28	5
	F. W.	68	2.15	100,000	1.00	3½
		73	1.90	100,000	0.55	3
		82	2.00	100,000	0.55	3½
III	B. R.	37	1.00	150,000	0.27	6
	H. S.	38	1.15	400,000	0.38	6

* Plus bile salts.

reduction in threshold was no greater than without bile salts in other instances, as for example with F. W.

In short, the reasons for the differences between our results and those secured by Wald still remain to be discovered. In the light of all the variation encountered, it is likely that Wald's two subjects may have been, by a curious statistical twist, extreme variates in an ordinary population distribution.

V. Recovery. The occasionally dramatic effectiveness of single doses has given the impression that visual recovery from an A-deficient diet is a matter of hours or perhaps days. Our previous experience showed that

it required at least 2 months after resumption of a normal diet with or without supplementation of vitamin A for a return to normal. Similar reports have been made by McDonald and Adler, and by Steffens, Bair and Sheard. Our present experiments further confirm these findings.

We were able to follow for over two months the history of 9 out of the 17 subjects who had been on a deficient diet, and who had returned to a normal diet containing about 10,000 units of vitamin A. During this subsequent normal period the subjects received a variety of treatments ranging from no supplements at all to generous additions of vitamins A, B_1, G, and C. The details of these supplements are given in the legend to figure 3.

Figure 3 shows that there is great variation among the subjects during recovery. Only 3 individuals (D. J., J. M., J. Mi.) recovered completely during the period of observation. We consider a recovery as complete when the threshold returns to within 0.3 log unit of the initial normal threshold; this is because a range of 0.3 log unit is not uncommon in the threshold of a single person measured over a long period of time. Of the remaining subjects, 4 (J. S., L. W., S. K., W. S.) were obviously on the road to recovery, while 2 (H. M., F. W.) showed a persistently elevated threshold throughout the three months of observation.

The most significant point which these data show is that *visual recovery from an A-deficient diet is a matter of weeks and months, and not of hours and days.* The fastest recovery was made in 6 weeks; the slowest was not complete after 3 months despite the fact that in one instance (F. W.) the supplementary daily dosage of vitamin A was raised to 100,000 units and that other vitamins were given in addition.

It is necessary to add that even these extremely slow individuals ultimately recover. A year after the experiment was terminated we were able to measure J. M. who had practically reached normal after 3 months and F. W. whose threshold had remained 1.2 log units above normal even after the same time. In the subsequent year both had become completely normal. In fact F. W's. threshold, which had been so alarmingly high, had dropped even below its original normal level.

The 4 subjects who recovered most rapidly illustrate the difficulties of drawing conclusions about the phenomenon. Of the two who recovered fastest (J. Mi., and D. J., 40 and 41 days respectively) one (J. Mi.) had received no supplements of any kind either during the A-deficient period or during the recovery period, while the other (D. J.) had received supplements of the other vitamins during the deficient period, and of A and the other vitamins during the recovery period. Of the other 2 subjects J. M. had received no supplements during the deficient period and 50,000 A units daily during recovery, while J. S. had received supplements of the other vitamins during the deficient period, and none during recovery (except

for the last few days). In other words, on the basis of these experiments one cannot find any effects of special dietary supplements on recovery rate. The recovery rate from a vitamin A-deficient diet almost seems an inherent property of the individual.

It is of course possible that we have missed some critical factor in all this procedure. We are inclined to doubt this because of the large variation shown by individuals during the period of the deficient diet. If individuals vary so much in their rate of depletion, it is not unreasonable to assume that they also vary in their rate of recovery. In any case, the important thing to emphasize is that recovery from vitamin A deficiency is a very slow process and that dramatic recoveries in short times are exceptions rather than the rule.

SUMMARY

1. Measurements of the dark adaptation of 17 young men made before, during, and after their stay on a vitamin A-deficient diet show that cone and rod visual functions are both affected by the diet. The cone thresholds rise less than the rod thresholds, but their behavior is approximately parallel. The best single criterion of these changes is the final rod threshold after 30 minutes in the dark.

2. When subjected to the deficient diet, most individuals (14 out of 17) responded by an immediate and unmistakable rise in visual threshold which continued for the duration of the diet. The other 3 subjects required 22, 55 and 60 days respectively before giving a clear and continuous rise in threshold. None of the subjects showed any skin symptoms, and only the two most extremely affected individuals recorded a subjective awareness of their raised visual thresholds.

3. Partial recovery of the visual threshold was accomplished in some individuals by single large doses of vitamin A. The extent of the recoveries was variable. The recoveries were never complete and were always temporary.

4. Permanent recovery from an A-deficient diet was slow, requiring weeks and months. The fastest subject returned to normal in 6 weeks; the slowest maintained a high threshold for over 3 months, but had recovered completely after a year.

5. Efforts to influence the visual responses during and after the A-deficient diet by supplementation with other vitamins yielded only negative results.

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THE EFFECT OF TRACES OF TIN ON THE RATE OF GROWTH OF GOLDFISH

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The nature of the rôle of tin in animal and plant physiology is not well known. Although tin has been reported from time to time as occurring in traces in the mammalian body (3, 4, 14), it has not been commonly regarded as important. Fearon (6) in his classification of the elements according to their relations with life includes tin among the variable micro-constituents; but Godden (8) concludes that there is no evidence to indicate that it exercises any useful function in animal nutrition. Richardson (13), on the other hand, regards tin, along with rubidium and aluminum, as accessory factors in animal nutrition, and adds that the physiological significance of these is still imperfectly understood.

The present report is an outgrowth of long and carefully executed laboratory studies on the rate of growth of goldfish in which it has been found that these animals grow somewhat, yet significantly, faster in water in which other goldfish have previously lived, for a limited period, than in otherwise uncontaminated water (1, 2). The last paper on this work (2) indicated that when the synthetic pond water, in which the fish lived, was made up with water from an aluminum-lined still, the growth results differed appreciably from those obtained when the water from a block-tin-lined still was used. Briefly, in the latter case, there was a greater growth of both the control fishes in uncontaminated water and of the experimental fishes in conditioned water, as against the growth of comparable groups in water from an aluminum-lined still; and also, the difference between the control and the experimental groups was significantly greater when the tin-lined still was in use.

In order to test the conclusion that tin, probably in ionic form, may have been responsible for these results, the present set of experiments was carried out. Here, stannous chloride was added in trace concentrations to the synthetic medium used for the fishes. This medium is a "synthetic pond water" developed in this laboratory for work with fishes and other aquatic forms. It consists of distilled water, in this case from an aluminum-lined still, to which salts of analytical quality are added in

following quantities per liter: 100 mgm. $CaCl_2 \cdot 4H_2O$, 50 mgm. $MgSO_4 \cdot 7H_2O$, 50 mgm. K_2SO_4, and 50 mgm. $NaNO_3$. It was found, empirically, that a concentration of $SnCl_2$ of the order of magnitude of 0.000005 M would not increase the specific conductance of stock distilled water (with a value of 2×10^{-6} reciprocal ohms) beyond our arbitrarily set upper limit for "good" distilled water of 4×10^{-6} reciprocal ohms. Concentrations of tin up to this strength might conceivably have been present in the water during experiments when the tin-lined still was in use.

The methods for handling the fish have been described in detail elsewhere (1, 2). In summary, immature single-tailed goldfish (*Carassius auratus*) ranging in length from 30 to 45 mm. were studied for their growth during brief assay periods, usually 20 days. Photographs taken before and after each period were measured and the *increase in length* is reported here in artificial units (1 unit = 0.82 mm.). The fish were kept singly in one-gallon, glazed earthenware jars, filled to 2 liters, and were changed daily to carefully washed similar jars containing freshly prepared water. The fish were usually fed during the last 3 hours of a 24 hour period so that, during each day, they were not in contact with food, except for the presence of minute regurgitated particles in the fish-conditioned water; otherwise they were in clean (food-free) water. The food consisted of ground dry Quick Quaker oats.

TABLE 1

Increase in growth of fish; analyzed by experiment

EXPERIMENT	CONCENTRATION 10^{-6} M	INCREASE IN LENGTH			NUMBER OF FISH	
(1)	(2)	Tin (3)	Control (4)	Difference (5)	Tin (6)	Control (7)
F20a*	4.2	0.73	0.41	0.32	9	10
F20b	4.2	1.17	0.45	0.72	9	9
F21a	4.2	0.89	0.85	0.04	9	10
F21b	4.2	1.22	1.04	0.18	10	10
F24a	4.2	2.56	1.69	0.87	15	14
F25a	5.0	0.75	0.61	0.14	19	18
F25a	2.5	0.43		−0.18	9	
F25b	5.0	0.72	0.60	0.12	17	19
F26a†	5.0	1.51	1.13	0.38	15	14
Mean		1.11	0.82	0.29	112	104

* "a" indicates that uncontaminated water was used as the medium, "b" that fish-conditioned water was used.

† Assay period of 14 days.

In some experiments, in connection with other work, fish conditioned water was used as a medium for the assay fish. The fish which were used to prepare this water were larger, averaging 70 mm. in length, and were kept in similar but larger jars which held 6 gallons of water, and these fish were also changed to fresh water daily and were fed in water other than that which they were conditioning.

The $SnCl_2$ was prepared in stock solutions of 0.01 M; this was added daily to the water to be conditioned and to the uncontaminated water, as the case may be, in appropriate amounts to secure the desired final concentration. No attempt was made to prevent the formation of stannic

TABLE 2

Increase in growth of fish; analyzed by paired fish

EXPERIMENT	CONCENTRATION 10^{-6} M	NUMBER OF PAIRS	INCREASE IN LENGTH	
			Sum of differences by experiment	Mean difference by experiment
(1)	(2)	(3)	(4)	(5)
F20a	4.2	9	2.6	0.29
F20b	4.2	8	5.7	0.71
F21a	4.2	9	1.2	0.13
F21b	4.2	10	1.8	0.18
F24a	4.2	14	11.1	0.79
F25a	5.0	17	3.1	0.18
F25a	2.5	8	−1.1	0.14
F25b	5.0	16	2.1	0.13
F26a	5.0	14	6.0	0.43
Total		105	32.5	
Mean			0.31	

ions, or to control the appearance of insoluble hydroxides by the use of acid since it was not desired to change the customary pH relations. The white suspension which began to appear in the stock solutions at about the 10th day of the experiment was simply stirred and added to the synthetic pond water as the stannous chloride had been previously.

The data were analyzed for statistical significance by "Student" 's method.[1] This was done in two ways: *a*, the mean differences in increase in length between treated and control fish were tabulated by experiment and the mean of means was analyzed; *b*, the differences between pairs of fish, randomly coupled within each experiment, were averaged and this

[1] Statistical significance is measured by P, calculated by "Student" 's method, which is designed for situations where relatively small numbers of observations are involved. P = 0.05 is equivalent to 2 × the standard error, or 3 × the probable error, and is taken as the upper limit of statistical significance (7, 16).

mean of differences was tested.[2] The results of the analysis by both the paired experiment and the paired fish methods are shown in tables 1 and 2, respectively. From the former it will be seen that in 9 comparisons, by experiment, the fish in tin-treated waters grew, on the average, 0.29 units more, in the indicated assay periods, than did the control fish in tin-free water, and this is significant for $P = 0.0316$. When the case of 2.5×10^{-6} M concentration is omitted, the difference between the tin-treated and the control fish becomes 0.35, and since $P = 0.0132$ this is even more significant.

In table 2, where the same data are analyzed by the paired fish method, similar results are obtained. Here, the over-all difference is 0.31 and, since $P = 0.000318$, is to be taken very seriously. Again, when the atypical group with the 2.5×10^{-6} M concentration is omitted, the difference, now 0.35, has an even more significant P value of 0.000096. This means that there are only 96 chances in a million that this result would be obtained by random sampling.

It should be stated at this point that only in experiments F20b and F24a of both tables, and also in F20a in table 2, did the differences show significance when analyzed within each experiment. However, it will be seen, by reference to column 5 in each table, that in 8 of the 9 experiments there was a positive trend, and that this trend is significant is indicated in the preceding paragraphs.

From these data it may be concluded that tin in one or other of its ionic forms, probably the stannous, promotes growth in goldfish under experimental conditions. This conclusion supports the inference made in a previous paper (2) that the change from a tin-lined to an aluminum-lined still may have affected the composition of the distilled water sufficiently to account for the observed changes in growth which accompanied the transfer of stills. Specifically, it seems quite possible that the tin-lined still gave off enough tin, evidenced by the observed rapid corrosion, to affect favorably the rate of growth of the fish, and the absence of traces of tin when the aluminum-lined still was in operation resulted in a decreased rate of growth under a variety of conditions.

That traces of tin may contaminate distilled water is suggested by the work of Stout and Arnon (15) who report that ordinary distilled

[2] In a the formula $t = \frac{\bar{x}}{\sqrt{\frac{\Sigma x^2 - \bar{x}\Sigma x}{n(n-1)}}}$ was employed; here x represents the mean difference in each experiment (column 5), $\bar{x}$ the mean of means, and n the number of experiments. In b the same formula was used, but here x is the difference in increase in growth for each randomly chosen pair, $\bar{x}$ the mean of such differences, and n is the number of pairs. P is obtained from a table of probabilities for "Student" 's distribution.

water contained a variety of metal contaminants, varying from 0.1 to 0.01 part per million, and which were traced to tin-lined copper stills and to the metal piping through which the distilled water circulated. Earlier, in 1933, Hance (9), using spectroscopic analysis, found traces of tin, as well as of zinc, copper, lead, nickel, and chromium, in the distilled water prepared at the Hawaiian Sugar Planter's Experimental Station.

The stimulating properties of tin for biological systems are not unknown. Micheels and De Heen (12) and again Micheel (11) found that colloidal tin stimulated the germination and early growth of wheat, oats, peas, and buckwheat. Yoshida (17, 18) reported that colloidal tin, as well as the colloidal preparations of other metals, accelerated the growth of fibroblasts and of splenic tissue *in vitro* when present in moderate concentrations, while it inhibited growth at higher and had no effect in lower concentrations. Hotchkiss (10), working with suspensions of *B. coli communis*, found that for a large number of salts of heavy metals, each salt was toxic at some concentration, but that in many of these salts a concentration was found which stimulated the growth of the treated bacterial suspensions over that of the untreated control. $SnCl_4$ was found to be stimulating at 0.00005 to 0.000005 M. Similarly, Young (19) found that tin stimulated the growth of timothy when present at 100 parts per million, and of algae when the concentration was between 0.002 and 0.004 part per million. Cohen (5) also found that tin in concentrations of 0.01 and 0.05 parts per million, as stannic chloride, stimulated root growth in sunflowers.

It will thus be seen that the bulk of the work on the stimulating properties of trace quantities of tin has been done in the fields of botany. The present paper indicates a possible rôle of tin in the metabolism and growth of fishes. Tissue cultures aside, so far as we know, there is no record of any previous work with tin with similar results on any of the classes of vertebrates.

SUMMARY

1. Tin, as stannous ion, in concentrations of the order of 0.000005 M, has been found to accelerate the growth of goldfish, during brief assay periods, over that of untreated control fish.

2. This report extends, for the first time, the stimulating properties of traces of tin to the growth of vertebrate organisms.

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GROWTH AND DEVELOPMENT OF SIX GENERATIONS OF THYMECTOMIZED ALBINO RATS[1]

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Andersen (1932) in reviewing her own experiments and the studies of other workers upon thymectomized animals concluded that there is little or no relationship between the thymus and the gonads. In most of these studies thymectomies were performed in animals at least 20 days old. However, Andersen stated that in some of her rats the thymus was re-removed on the first day of life. Extensive data on these latter animals were not presented but the comment was made that no effects on vaginal canalization or development were noted.

Shay and his co-workers (1939) have since reported that irradiation of the thymus area in the 1 or 2 day old rat with massive doses of x-ray produced complete atrophy of the thymus. They also reported a retardation of growth in the males, an absence of sperm from the testes in animals 100 days old, and the presence of castration cells in the pituitary. However, if x-rayed males were permitted to live for 200 days they were essentially normal, i.e., spermatogenesis was resumed and castration cells disappeared from the pituitary.

Hashimoto and Freudenberger (1939) have criticized this work, but their data presented on thymectomized animals fails to clarify the issue since they used older animals (25 days). They feel, as we do (Nelson, 1939) that the thyroids must have been damaged by secondary irradiation in the experiments of Shay. It is unlikely that the thyroids were completely destroyed, since that would lead eventually to a complete cessation of growth (Salmon, 1938), but it seems probable that they were damaged enough to produce thyroidectomy cells which appear to be identical with castration cells (Nelson and Hickman, 1937; Severinghaus, 1937).

Dr. J. C. Plagge of the University of Chicago has kindly informed us that he has been unable to find any differences in the time of appearance of sperm heads between normal or sham operated controls and rats thymectomized at 1 day of age. Growth of both soma and gonads was

[1] This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

unaffected by the operation, a finding which held as well for the hormone output of the testis.

Putzu reported in 1934 that thymectomy in 5 successive generations of rabbits had no effect on growth or development. However, Einhorn and Rowntree (1936a, b; 1937) stated that thymectomy in successive generations of albino rats resulted in an accruing retardation of growth and development. This will be recognized as the reverse of results reported by these workers in successive generations of albino rats treated with

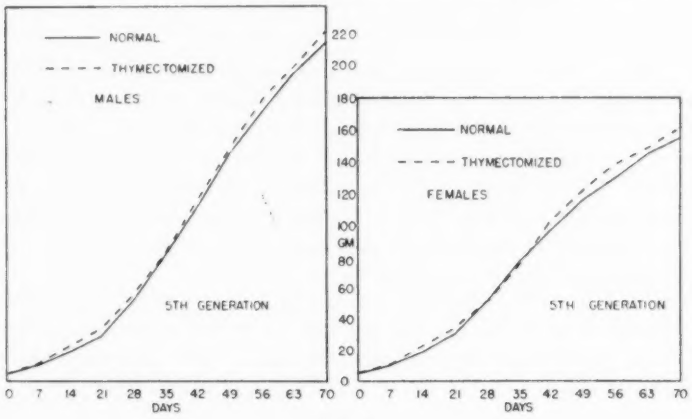

Fig. 1

TABLE 1

Development

SERIES	VAGINAL CANALIZATION			1ST ESTRUS	TESTES DESCEND	EARS OPEN	INCISORS ERUPT	EYES OPEN
	Weight	Body length	Days					
	grams	*mm.*		*days*	*days*	*days*	*days*	*days*
Normal.....	85 ±16.2	137 ±10.9	40 ±5.3	40 ±5.5	19 ±1.4	2.5	11.0	15.8
Thymectomy.	94 ±16.6	146 ±10.2	40 ±5.7	41 ±5.5	17 ±1.4	2.5	10.3	14.4

thymus extract (Hanson). Thymectomized animals which were treated with the extract grew normally or showed a slight acceleration (1938).

Chiodi (1938a, b) has reported that thymectomy in five successive generations of albino rats failed to alter the course of growth or development.

EXPERIMENTAL. All members of a litter of albino rats were thymectomized at 21 days and weaned at 25 days. This litter was used to initiate the experimental series. Thymectomies were performed uniformly at 21 days by the usual sternal approach. The method was essentially that of Einhorn (1936a), with the addition of tying in the end of the

thread for closure of the thorax before making the sternal incision. The operative mortality was approximately 5 per cent. Control animals were housed in adjacent cages under rigidly standardized conditions and were inbred to the same extent and over the same period as the experimental animals. All observations were made and recorded at the same time each day. The animals were fed our stock diet of Ti-O-Ga dog chow and water with the semi-weekly addition of lettuce. Animals which were not reserved to carry on the next generation were sacrificed at 70 days and the viscera were carefully weighed.

Although 6 generations have been studied, the extensive data for the 5th generation only are being presented since the larger number of animals

TABLE 2

Body weights and dimensions @ 70 days

MALES	NUMBER	WEIGHT	BODY	TAIL	FORE LIMBS	HIND LIMBS
		grams	*mm.*	*mm.*	*mm.*	*mm.*
Normal	25	216 ±21	195 ±6	165 ±9	160 ±4	210 ± 6
Thymectomy	17	225 ±13	197 ±5	171 ±7	152 ±11	203 ±13
Females						
Normal	23	155 ±13	179 ±7	154 ±7	154 ± 6	195 ±13
Thymectomy	24	161 ±14	178 ±5	159 ±4	139 ±10	182 ±16

TABLE 3

Organ weights @ 70 days. Weights in milligrams

MALES	THYMUS	KIDNEY	ADRENALS	THYROID	PITUITARY	HEART	TESTIS	SEMINAL VESICLE	
								Full	Empty
Normal	477 ±68	1,050 ±156	35 ± 5	18 ±4	8.4 ±1.2	785 ±89	1,235 ±146	298 ±60	136 ±40
Thymectomy		977 ±138	39 ±10	20 ±6	8.8 ±1.9	770 ±51	1,301 ± 93	295 ±67	144 ±30
Females							Ovaries		
Normal	378 ±69	787 ± 98	45 ±10	17 ±3	9.8 ±1.8	612 ±58	59 ± 15		
Thymectomy		667 ± 79	44 ±17	16 ±3	8.3 ±1.2	563 ±64	58 ± 11		

in this group gives the data a greater statistical significance. However, these figures for the 5th generation might well apply to any of the other 5 generations.

An examination of the growth curves in figure 1 will show the complete absence of any retarding effect of thymectomy upon the rate of growth. Indeed, the thymectomized animals, both males and females, were slightly larger than their corresponding controls.

The absence of differences in the occurrences of developmental events is shown in table 1. The fact that the body length at vaginal canalization shows the smallest proportionate standard deviation of any of the criteria measured argues in favor of the reliability of the observations since Engle

and his associates have pointed out that of all the criteria which can be measured at vaginal canalization, body length show the least deviation.

The body weights and dimensions and the organ weights at 70 days (tables 2 and 3) for experimental and control animals show no differences as great as one standard deviation.

SUMMARY

Thymectomy on the 21st day of life in six successive generations of albino rats has not altered the rate of growth in males or females. Furthermore, in none of the developmental events which we have studied has there been any departure from the normal time of occurrence. In all, 69 males and 60 females were thymectomized, while 124 males and 139 females were studied as controls.

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CHANGES IN RESPIRATION ON INFLATION AND DEFLATION OF THE CHEST

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In one of the more recent studies of the effects of inflation and deflation of the chest (Hammouda and Wilson, 1932) alterations of pulmonary volume were obtained by varying the pressure around an animal in a body plethysmograph. The conclusions of these authors differ in several respects from those of previous workers. The observations to be reported in this paper were made by a similar method. They do not, however, support the more distinctive conclusions of Hammouda and Wilson as to the functions of afferent fibers in the vagus nerves. The difference in our conclusions is due in part to the introduction of another method of analyzing the reflex effects of the vagi, making use of measurements of resistance to inflation and deflation of the chest. In addition, some information is presented as to the effect of vagotomy on the carbon dioxide and oxygen content of arterial blood which has a bearing on the interpretation of the effects of vagotomy.

METHODS. In all experiments cats were used, either decerebrated under ether at a level just above the superior colliculi, or lightly anesthetized with chloralose and urethane (40 mgm. and 0.4 gm. per kgm. respectively), supplemented with ether during the preparatory operations. On two occasions Dial was used intraperitoneally. After tracheotomy, isolation of the vagi and (when blood pressure was to be recorded) cannulation of one carotid, the animals were placed in a body plethysmograph. Air-tight closure around the base of the neck was secured with plaster of Paris. An outlet from the plethysmograph was connected, as illustrated in figure 1, with a 20-liter bottle and with a mercury manometer by which the degree of inflating or deflating force was controlled. Sometimes the manometer was arranged to record the inflating or deflating pressure. The purpose of the large bottle was to minimize the changes of pressure in the plethysmograph during the animals' respiratory movements. Breathing was recorded by a spirometer of the Krogh type, connected through a soda lime cannister to the tracheal cannula. Oxygen was bubbled into the closed system just fast enough to replace that consumed. This arrangement allows the quantitative measurement of a,

the tidal air; *b*, the immediate change in lung volume on vagotomy; *c*, the change in lung volume on inflation or deflation; *d*, the resistance to inflation and deflation, which can be expressed as the ratio of applied pressure to the change in lung volume.

In the decerebrate animals, both carotids were tied, thus securing uniform stimulation of the receptors of the carotid sinus region before and after vagotomy. The possibility of pressure changes in the aortic region affecting the results is not eliminated, nor is the effect of changes of blood pressure acting on the medulla.

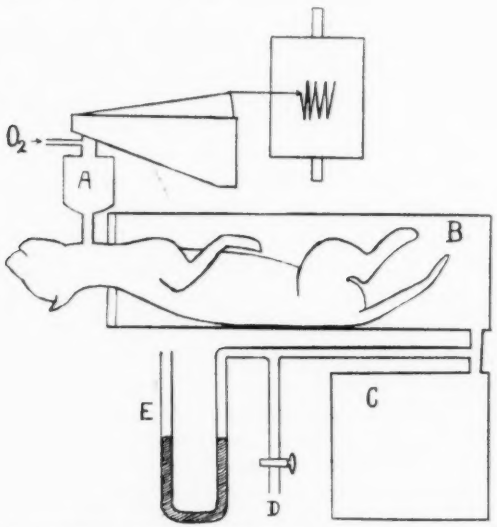

Fig. 1. *A* is a cannister containing soda lime connecting a tracheal cannula in the cat with the spirometer; *B* is the body plethysmograph; *C*, a twenty-liter bottle; *D*, a side-arm for the application of suction or pressure; *E*, a manometer.

RESULTS. The results most typical in this series will be presented first. Interesting but irregular results will be dealt with for the most part in the discussion.

Typical effects of inflation and deflation before and after vagotomy are shown in figures 2 and 3. With sufficient inflation there was as a rule a pause in the breathing, during which a progressively mounting expiratory effort was very evident on the tracing. This was terminated abruptly by a vigorous inspiratory effort. The cycle then repeated itself with increasing frequency, in consequence no doubt of the inadequate pulmonary ventilation.

On deflation of the chest there was in most experiments (12 out of 15) a

prompt increase in frequency. In the most sensitive of the animals a decrease of 5 cc. in a cat weighing 2.2 kgm. was sufficient to double the respiratory rate.

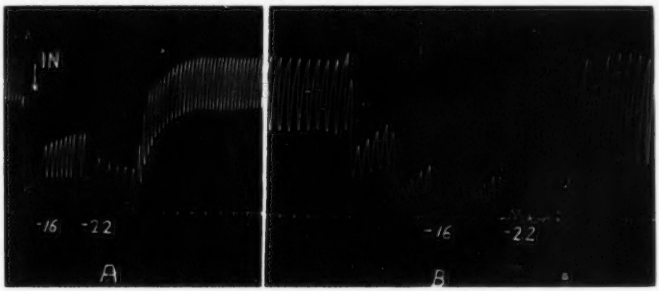

Fig. 2. Showing the respiration and effects of inflation of the chest of cat C (decerebrate), A about five minutes before and B about five minutes after double vagotomy. −16 and −22 refer to the negative pressure in the plethysmograph in millimeters of mercury. Note the greater lung volume during distention with the same negative pressure after vagotomy. Time interval, 5 sec.

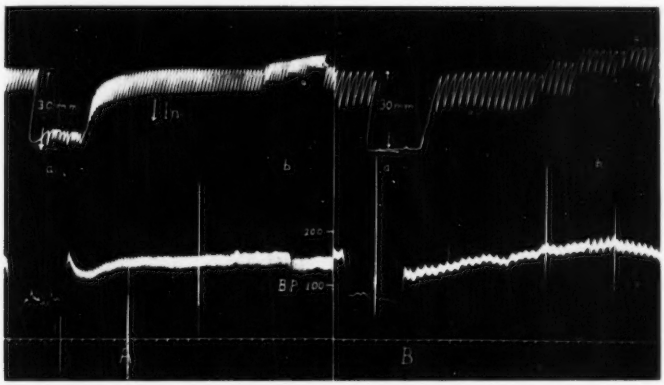

Fig. 3. Tracing from cat B (decerebrate), shows in addition, the effects of deflation of the chest and a record of blood pressure. A was taken about three minutes before and B about three minutes after vagotomy. Little *a* marks the period of application of a negative pressure of −22 mm. Hg., both before and after vagotomy. Little *b* marks compression of the chest by successive application of 5, 10, and 15 mm. Hg positive pressure both before and after vagotomy.

After vagotomy the following changes are demonstrated in figures 2 and 3. 1. The lung volume at the end of expiration is unchanged (fig. 5). (This was true in nine out of ten experiments.) 2. The respiratory rate is slower. Only two cats which were rather deeply anesthetized failed to

show this change. 3. No pause in breathing follows inflation. 4. The shape of the tracing during inflation suggests decreased expiratory effort. 5. The resistance to inflation is less. 6. On deflation no increase in frequency is evident, nor did it occur in any experiment. 7. The resistance to deflation is also less. 8. The speed of inspiration within two minutes after vagotomy is definitely slower than before vagotomy (fig. 4). This effect was observed in four out of eight experiments; in the other four no definite change was demonstrable.

The observation that vagotomy often decreases the speed of inspiration substantially confirms the findings of Gesell, Steffenson and Brookhart (1937). Hammouda and Wilson, however, report that immediately after vagal section the inspirations are deeper and faster, only settling

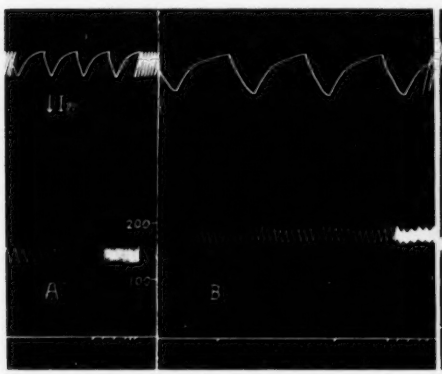

Fig. 4

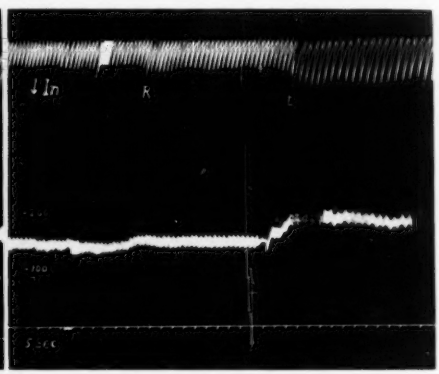

Fig. 5

Fig. 4. A faster record showing a decreased speed of inspiration (less slope) after vagotomy. A two minutes before, B two minutes after vagotomy.

Fig. 5. Shows the constancy of lung volume at the end of expiration of cat B after cutting the right vagus, *R*, and the left vagus, *L*, in the neck.

down to their previous speed after about half an hour. It seemed important to determine the part played by the level of the chemical stumulus after vagotomy in these variable results.

Blood gases after vagotomy. Five animals were used, three decerebrate and two anesthetized with chloralose and urethane. They were prepared as in other experiments by tracheotomy, isolation of the vagi and cannulation of one carotid artery. Four samples of arterial blood were taken at twenty to twenty-five minute intervals, two before vagotomy and two after, and analyzed for carbon dioxide and oxygen in a Van Slyke apparatus. In every instance the arterial CO_2 content was higher ten minutes after vagotomy than it was ten minutes before vagotomy. The smallest rise was 0.3 v.p.c., the largest 2.5 v.p.c. Consequently it may

be assumed that the slower speed of inspiration after vagotomy could hardly have been due to a lower arterial CO_2 content. The cases in which no definite change in the speed of inspiration could be detected may well have had sufficient rise in arterial CO_2 to offset the tendency of vagotomy to decrease the speed of inspiration. In the second blood sample taken after vagotomy (i.e., one-half to one hour after vagotomy) the CO_2 had, in all cases but one, fallen. It is hard to say, without further experiment,

TABLE 1

Effects of vagotomy on the gases of the arterial blood

	TIME IN MINUTES				
	0	29	42	55	79
Cat 1, anesthetized					
CO_2 v.p.c.	29.2	29.5	Vagotomy	32.1	33.5
O_2 v.p.c.	18.3	17.5	Vagotomy	18.2	17.7
	0	25	34	47	65
Cat 2, decerebrate					
CO_2	30.6	28.7	Vagotomy	29.5	22.5
O_2	18.2	15.8	Vagotomy	14.8	14.5
	0	30	40	50	65
Cat 3, decerebrate					
CO_2	27.0	23.9	Vagotomy	25.6	25.1
O_2	17.2	16.2	Vagotomy	15.7	15.7
	0	22	27	44	99
Cat 4, decerebrate					
CO_2	36.0	37.7	Vagotomy	38.9	35.0
O_2	15.6	15.1	Vagotomy	15.4	14.7
	0	28	40	50	74
Cat 5, anesthetized					
CO_2	44.8	43.7	Vagotomy	44.0	42.1
O_2	16.2	15.7	Vagotomy	15.7	15.4

whether this delayed fall in arterial CO_2 is due to overventilation or to circulatory or metabolic effects of vagotomy. The O_2 content of the blood declined slightly in all the series, the last sample containing 1 to 3 v.p.c. less oxygen than the first.

The great increase in speed and depth of inspiration after vagotomy in Hammouda and Wilson's experiments may have been due to the use of morphine, since according to Henderson and Rice (1939) one of the effects of morphine is to augment the effectiveness of the vagal impulses

inhibitory to inspiration. The relation of changes of blood pressure to these results will be considered later.

Resistance to inflation and deflation. In figures 2 and 3 are shown the changes in lung volume produced by equal pressure changes before and after vagotomy. After vagotomy the changes in lung volume to both inflating and deflating pressures are definitely greater. It is difficult to interpret these effects otherwise than as due to removal of active resistance to deflation (by inspiratory muscles) and active resistance to inflation (by expiratory muscles). For example, it is difficult to see how increased inspiratory activity due to release of inhibition by the vagi could possibly explain a decreased resistance to deflation, as well as to inflation. Nor would the invocation of decreased inhibition to both inspiration and expiration explain the facts any better. Speaking strongly for an augmentor influence on expiration transmitted by the vagi during inflation, is the greater amplitude of breathing during inflation before vagotomy, whereas at normal lung volume the amplitude is greater after vagotomy.

After pithing the brain and cord there is a still further slight decrease in resistance to inflation, but not to deflation. In addition, there is a loss of elasticity of the chest in the sense that the chest does not quite return to its original volume after distortion. Apparently after complete denervation the chest is, within certain narrow limits (e.g., 25 cc. in a 3 kgm. cat) plastic. It stays at the volume at which it is set.

Discussion. The reactions of the completely denervated chest raise a question as to the propriety of the term "passive expiration." It seems hardly proper to apply the term passive to an expiration in which tonically active muscles participate. And it seems even less proper to apply it if the tonically active muscles have been reciprocally inhibited during inspiration. Perhaps an even more important reason for abandoning the term "passive expiration" is that while it is easy enough to distinguish expirations of varying degrees of activity, it is a practical impossibility to determine that any expiration is purely passive, i.e., unassociated with any increased activity in any expiratory muscle.

Hammouda and Wilson (1932) conclude that "there is no evidence of an immediate active expiratory response to expansion or an inspiratory response to collapse implied in the Hering-Breuer hypothesis." This statement is perhaps understandable in view of these authors' preoccupation with the phenomena of increased frequency on deflation and decreased frequency on inflation, but can hardly be accepted. Examination of their own tracings for changes of resistance to inflation on vagotomy gives the evidence which they have overlooked. Even casual inspection of their tracings suggests a definite difference in the character of expiration during inflation, before and after vagotomy. They appear to have been misled by two conditions of their experiments. 1. The use of progressively increasing pressures for expansion and compression, instead of steady

pressures. 2. The occurrence of definite changes in lung volume on vagotomy in their published tracings.

Changes in lung volume on vagotomy. In this series in only one experiment out of ten was there a change in lung volume after vagotomy which lasted more than one minute. In that experiment the lung volume at the end of expiration was increased for an indefinite period. In most of the other experiments only a transitory increase or decrease lasting for only two or three breaths was observed. The tracings of Hammouda and Wilson show in one case a considerable increase, in the other a considerable decrease after vagotomy. Head (1889) describes prolonged tonic contraction of the diaphragm after vagotomy as typical, but not invariable. The state of the chest or degree of expansion of the lung immediately prior to section is not always specified and must play an important part (Hess, 1936). The fact that no change of lung volume occurred on vagotomy in most of these experiments must mean that either no change occurred in tonus of the respiratory muscles or that changes of equal effectiveness occurred in the expiratory and inspiratory muscles. This is important in the interpretation of the larger lung volume at the end of expiration during inflation after vagotomy, which might be attributed to decreased activity of expiratory muscles or to relatively increased activity of inspiratory muscles at the end of expiration. The latter explanation is incompatible with the finding of unchanged lung volume at the end of expiration when under no distorting pressure.

It is not suggested that active expiration is entirely dependent on afferent vagal impulses. Adrian (1933) has noted its presence after vagotomy, as evidenced by action currents in expiratory muscles. Evidence to the same effect has been obtained in experiments in this series by attaching a Cushny recorder to the rectus abdominis muscle. Rhythmic inhibition and activity were not easily detected during quiet breathing but were very evident during asphyxia. Similar expiratory activity was observed during asphyxia after vagotomy. There was, however, a very definite delay after the cessation of inspiration before the onset of the expiratory contraction as contrasted with an immediate sequence of expiration after inspiration before vagotomy.

In view of the suggestion of Hammouda and Wilson that the increased frequency of breathing on deflation was due to tonic impulses augmentor to frequency acting on a pneumotoxic centre when these impulses were opposed by fewer inhibitory impulses generated by stretching of the lungs, it seems important to point out that the increased frequency is not an invariable response to deflation, even when the vagal reflexes are not depressed at all by anesthesia. Sometimes slowing occurs, or slowing followed for no apparent reason by increased frequency or vice versa. In one case mild deflation produced inspiratory spasms or apneuses.

Adrian (1933) has supported very plausibly the hypothesis that most

of the phenomena attributed to afferent vagal impulses might be explained by postulating only impulses inhibitory to inspiration and evoked by inflation of the lungs. It is probably futile to attempt to distinguish between impulses directly inhibitory and reciprocally inhibitory but it seems important to reiterate that the experiments reported in this paper allow the inference of impulses directly augmentor to inspiration and expiration (and hence of reciprocal inhibition) with more certainty than the inference of independent inhibitory action.

Schmidt (1932) has shown that changes in blood pressure can cause changes in respiration by their effects on blood flow through the medulla as well as by their effects on receptors in the aortic and carotid sinus regions. Vagotomy in these decerebrated animals has usually resulted in a considerable rise in blood pressure (30 or 40 mm. Hg). The effect of such a rise would be to depress respiration even after denervation of the carotid and aortic arch receptors, and might be an important factor in explaining the decreased velocity of inspiration after vagotomy. Till this factor is properly controlled the existence of the proprioceptive impulses augmentor to inspiration postulated by Gesell, Steffanson and Brookhart to arise during inflation must remain in question.

Inflation of the chest in our experiments caused a large fall in blood pressure (fig. 3), while deflation caused a smaller rise. It seems unlikely that on deflation the rise in blood pressure (since it is in general terms depressant to respiration) could be in any part responsible for the greater inspiratory activity evident while the vagi were intact. On the other hand, it seems quite possible that during inflation the fall in blood pressure might well be in part responsible for the greater expiratory activity observed while the vagi were intact.

SUMMARY

The effects on respiration of inflation and deflation of the lungs in decerebrate and anesthetized cats have been recorded mechanically, before and after vagotomy. The changes after vagotomy include 1, decreased resistance to inflation; 2, decreased resistance to deflation; 3, decreased speed of inspiration.

Vagotomy results in a small rise in arterial CO_2 followed in about an hour by a fall.

The probable effects of changes in blood pressure are discussed in relation to the results as a whole.

The results are interpreted as evidence of the importance of the afferent influences of the vagi, postulated by Hering and Breuer to be augmentor to expiration on inflation and augmentor to inspiration on deflation.

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CEREBROSPINAL FLUID PRESSURE AND VITAMIN A DEFICIENCY[1]

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In previous publications Moore, Huffman and Duncan (1935) and Moore (1939a, 1939b) reported a type of blindness resulting from a constriction of the optic nerve as due to a deficiency of vitamin A in the ration of the bovine. Blindness was preceded by papilledema, nyctalopia, incoördination and syncope and a decrease in the carotene content of the blood plasma. In private communications several investigators have since reported papilledema in calves fed vitamin A deficient rations. The presence of papilledema is usually considered prima facie evidence of an elevated cerebrospinal fluid pressure. Since some of the manifestations accompanying this syndrome, particularly the papilledema, syncope, and incoordination, might be accounted for by the presence of an increased cerebrospinal fluid pressure, pressures were determined on young bovine fed a vitamin A deficient ration and are reported in this paper.

EXPERIMENTAL. Blood plasma carotene determinations were made each week according to a recently published method by Moore (1939c). For the carotene determinations of the alfalfa meal, the method of Guilbert as modified by Peterson et al. (1937), was used.

The low carotene ration consisted of skimmed milk and a concentrate mixture consisting of 240 pounds barley, 180 pounds rolled oats, 180 pounds wheat bran, 60 pounds linseed oil meal and 8 pounds salt. This ration contained sufficient carotene to supply two to four micrograms per kilogram of body weight per day. Wood shavings were used as bedding.

Cerebrospinal fluid pressures were obtained by puncture into the subarachnoid space. The insertion into the subarachnoid space was made through the dorsal opening in the atlanto-occipital articulation. No anesthetic was used and the records were obtained with the animals in the standing position.

The animals used were of the Holstein breed and were for the most part the same individuals described in previous reports by Moore (1939a, 1939b). The principal plan was to limit the carotene intake till a change in cerebrospinal fluid pressure occurred and then to add a supplement in

[1] Journal article 421 (n.s.) Michigan Agricultural Experiment Station.

an attempt to restore the pressure to normal. At the same time ophthalmoscopic observations were made at appropriate times and the animals were tested for night blindness by attempting to run them into objects and observing their behavior in dim light, a method similar to that used by Guilbert and Hart (1935). The animals were weighed every 10 days at which time adjustments were made in the amount of carotene supplement fed. Data on only three of the experimental animals are presented. These data are representative of the group as a whole.

Animal 1 was a Holstein male which had been receiving since 179 days of age a carotene supplement in the form of alfalfa leaf meal supplying 60 micrograms of carotene per kilogram of body weight per day. At 598 days the supplement was removed from the ration. At this time the animal showed no fundus changes and appeared normal and active. The cerebrospinal fluid pressure was also normal as shown in graph I. At 663 days this animal showed night blindness and the cerebrospinal fluid pressure rose from a normal of 110 mm. of saline to 200 mm. A slight edema of the nerve head developed at 709 days of age at which time pressure had increased to between 250 and 300 mm. of saline. At 720 days the tapetum lucidum was quite bleached of its normal yellow color and there was slight edema at the edges of the nerve head. A small hemorrhage was noticed on the nerve head of each eye and the capillaries were distended. The calf showed diarrhea, some incoördination and swelling at the hocks. At 721 days alfalfa supplying 120 micrograms of carotene per kilogram of body weight was added to the ration. The cerebrospinal fluid pressure decreased, plasma carotene increased and the night blindness disappeared. However, the cerebrospinal fluid pressure did not return to normal even after 109 days of supplemental feeding although the slight edema of the nerve head had almost disappeared. At this time the animal had recovered from the effects of the deficiency as judged by external appearance and was disposed of because he was too large to manage effectively. At autopsy no gross changes were noted.

Animal 2 had received crystalline carotene dissolved in cottonseed oil since 109 days of age at the rate of 300 micrograms per kilogram of body weight. At 178 days the level was reduced to 120 micrograms. At 498 days of age when the animal appeared in normal health and no fundus changes could be noticed the carotene supplement was removed from the ration. Nyctalopia and papilledema developed at 536 days. At 563 days the cerebrospinal fluid pressure had increased from 115 mm. to 250 mm. of saline and both eyes showed a choking of 3 diopters. At 568 days the supplement was again added to the ration. Night blindness disappeared in about 6 days, and the plasma carotene showed an immediate increase. The cerebrospinal fluid pressure also dropped but remained above normal. Graph II shows the changes which occurred in this calf. It was necessary

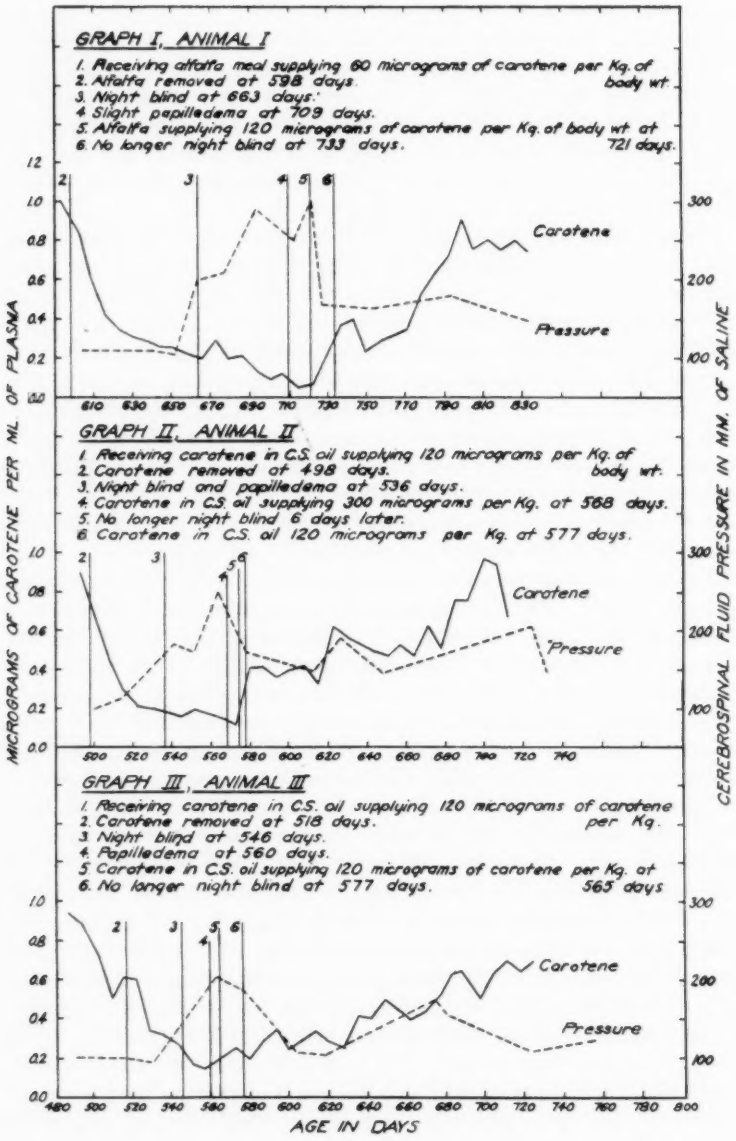
GRAPH I, ANIMAL I
1. Receiving alfalfa meal supplying 60 micrograms of carotene per Kg. of body wt.
2. Alfalfa removed at 598 days.
3. Night blind at 663 days.
4. Slight papilledema at 709 days.
5. Alfalfa supplying 120 micrograms of carotene per Kg. of body wt. at 721 days.
6. No longer night blind at 733 days.
Carotene
Pressure
GRAPH II, ANIMAL II
1. Receiving carotene in C.S. oil supplying 120 micrograms per Kg. of body wt.
2. Carotene removed at 498 days.
3. Night blind and papilledema at 536 days.
4. Carotene in C.S. oil supplying 300 micrograms per Kg. at 568 days.
5. No longer night blind 6 days later.
6. Carotene in C.S. oil 120 micrograms per Kg. at 577 days.
Carotene
Pressure
GRAPH III, ANIMAL III
1. Receiving carotene in C.S. oil supplying 120 micrograms of carotene per Kg.
2. Carotene removed at 518 days.
3. Night blind at 546 days.
4. Papilledema at 560 days.
5. Carotene in C.S. oil supplying 120 micrograms of carotene per Kg. at 565 days
6. No longer night blind at 577 days.
Carotene
Pressure
MICROGRAMS OF CAROTENE PER ML. OF PLASMA
CEREBROSPINAL FLUID PRESSURE IN MM. OF SALINE
AGE IN DAYS

Fig. 1

to dispose of the animal at 733 days of age. At this time the papilledema had decreased to about 2 diopters, and its presence was confirmed at autopsy. The ventricles of the brain were also considerably distended being 3 to 4 times their normal size and the choroid plexus appeared edematous.

Animal 3 had been receiving crystalline carotene dissolved in cottonseed oil at the rate of 120 micrograms of carotene per kilogram of body weight since 188 days of age. At 518 days of age when the animal was apparently in good health and showed no ocular changes the crystalline carotene was removed from the ration. The cerebrospinal fluid pressure at this time was 100 mm. At 546 days as shown in graph III this animal was night blind and at 560 days showed edema of the nerve head, bleaching of the tapetum lucidum, considerable exophthalmus and some incoördination. At this time the cerebrospinal fluid pressure had increased to 200 mm. of saline. At 565 days the crystalline carotene supplement was added to the ration. When examined with the ophthalmoscope at 572 days the right eye showed a choking of 3 diopters and the nerve margins were blurred. There were about 10 petechial hemorrhages on the nerve head. These hemorrhages had largely disappeared a week later or after about 2 weeks of supplemental feeding. At 577 days the animal was no longer night blind. The cerebrospinal fluid pressure decreased to normal but later showed two higher values and then again returned to the normal level. The edema of the nerve head gradually decreased also.

Discussion. The evidence presented indicates that a deficiency of vitamin A in the ration of the bovine permits an elevated cerebrospinal fluid pressure to develop. The results on all the animals show definitely that vitamin A deficiency and a low plasma carotene is correlated with a raised cerebrospinal fluid pressure. When vitamin A was withdrawn from the ration plasma carotene decreased and the pressure increased. When the deficiency was corrected the plasma carotene increased and the cerebrospinal fluid pressure decreased. The results with animals 2 and 3 afford almost indisputable proof of this relationship since the effect was produced by removing crystalline carotene from the ration and was alleviated by supplementing the ration with carotene. It will be noted that in only one instance (animal 3) did the cerebrospinal fluid pressure completely return to the normal level. Apparently the disturbances which are produced by vitamin A deficiency are slow in repair. It would appear to be a matter of 4 or 5 months. Unpublished observations on mature animals in studying the rate of decrease of papilledema also indicate a long period of recovery even when the animals are turned to pasture.

It will be noted that there were some variations in the time of first appearance and amount of papilledema and the development of cerebrospinal fluid pressure. Animal 1 showed a very high cerebrospinal pressure

and yet never developed a marked papilledema. These variations seemed to be more pronounced as the animals grew older. In unpublished observations on mature cows depleted of vitamin A, incoördination and syncope were present without marked fundus changes. If left on the ration sufficiently long, however, in most cases the fundus changes would finally occur. One would conclude, therefore, that the papilledema is much more easily produced in young animals. These differences might be explained by changes in intraocular tension. Studies should be made on mature animals concerning the relationship of the development of increased cerebrospinal fluid pressure and fundus changes. However, considerable difficulty is encountered in making repeated pressure measurements with larger animals.

In general the data on these calves show a rather definite correlation between papilledema and an elevated pressure, and it would seem reasonable to suggest that the papilledema is a direct result of the elevated cerebrospinal fluid pressure. It would also be possible to explain the papilledema as due to pressure on the optic nerve caused by stenosis of the optic canal, a condition which develops if the deficiency persists for long periods (Moore, 1939a, b). That the latter is not the true explanation, is indicated by the fact that papilledema occurs in mature cows due to vitamin A deficiency without any apparent stenosis of the optic canal.

In two of the calves (1 and 3) incoördination developed when the cerebrospinal fluid pressure had increased considerably and when the fundus changes were most pronounced. The incoördination rapidly disappeared when vitamin A was added to the ration coincident with the fall in pressure. This likewise suggests that the incoördination observed in vitamin A deficiency in the bovine may be related to cerebrospinal fluid pressure changes. On the other hand, the incoördination may be due to myelin degeneration. This was not determined. However, in pigs incoördination in vitamin A deficiency may not be correlated with myelin degeneration according to work reported by Eveleth and Biester (1937) and Bessey and Wolbach (1938) have expressed some doubt as to a relationship between myelin degeneration and vitamin A deficiency. One would not suspect that a condition of syncope (Moore, 1939a) was associated with myelin degeneration.

The question as to whether vitamin A deficiency produces similar changes in other species is interesting. Preliminary data with dogs indicate fairly conclusively that increased cerebrospinal fluid pressure develops on vitamin A deficient rations but that the fundus changes do not readily occur. Mellanby (1938) has noted widespread nerve degeneration in young dogs fed diets deficient in vitamin A and rich in cereals. Complete deafness was present in some of the animals which he attributed to nerve degeneration due to an overgrowth of bone of the labyrinthine cap-

sule. He expressed the belief that the degeneration of other nerves might be due to pressure caused by overgrowth of bone in the various foramina. It is suggested that the changes reported by Mellanby (1938) are related in some manner to those reported in calves where a constriction of the optic nerve develops.

CONCLUSIONS

1. A deficiency of vitamin A in the ration of the young bovine produces an increased cerebrospinal fluid pressure.

2. The increase in cerebrospinal fluid pressure is accompanied by papilledema, nyctalopia, syncope and incoördination.

3. On return to a normal diet the cerebrospinal pressure slowly returns to normal, while the quoted disturbances disappear.

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THE RECEPTIVE FIELDS OF OPTIC NERVE FIBERS

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Appreciation of the form of the retinal image depends upon a correspondence between the distribution of light on the retina and the distribution of activity among the fibers of the optic nerve. This correspondence may be studied directly by recording the activity in single optic nerve fibers in response to illuminating various parts of the retina.

A given optic nerve fiber responds to light only if a particular region of the retina receives illumination. This region is termed the receptive field of that fiber. In a previous paper describing the responses in single optic nerve fibers from the cold-blooded vertebrate eye (Hartline, 1938) it was noted that the receptive fields of the optic nerve fibers are of small but appreciable extent, and that their locations on the retina are fixed. It is the purpose of the present paper to describe further the characteristics of receptive fields, and to discuss some of the spatial factors involved in the excitation of the fibers of the optic nerve.

METHOD. The method for recording the activity in single optic nerve fibers from the eyes of cold-blooded vertebrates has been described in the previous paper (loc. cit.). An eye is excised, cut open, and small bundles of optic nerve fibers are dissected from the anterior surface of the exposed retina. The action potentials in these bundles are amplified and recorded with an oscillograph. When such a bundle has been split successfully, until only a single active fiber remains, the retina must be searched with a small spot of light to determine the region supplying that fiber. This search is aided by noting the direction, on the retina, from which the nerve fibers in the small bundle come, and by using large spots of light at first to locate the approximate position of the sensitive region.

The optical system employed in these experiments has likewise been described. A spot of light of suitable size is projected upon the exposed retina; the coördinates of its position, referred to an arbitrary point of origin on the retina, are obtained from readings of crossed micrometers which control its location. The micrometer readings are reduced to millimeters on the retina by multiplying them by the magnification of the optical system (0.32 or 0.15). Sharpness of focus of the spot on the retina

is checked in every experiment by direct observation through a dissecting microscope.[1] This optical system can provide a maximum intensity of illumination on the retina of $2 \cdot 10^4$ meter candles, which may be reduced to any desired value by means of Wratten "Neutral Tint" filters.

Eyes from large frogs (R. catesbiana), and from a few alligators, were used in the present study. In none of these experiments did the receptive fields of the fibers lie in or near the *area acuta* of the retina; this paper is therefore concerned only with properties of the peripheral retina. The preparations were always allowed 20 to 30 minutes for dark adaptation (at 25°C.), and observations were checked whenever possible to guard against slow changes in sensitivity.

RESULTS. The sensitivity of different regions of the retina to light must be defined with respect to the particular optic nerve fiber which is under observation. A spot of light in one location on the retina may elicit a vigorous discharge of impulses in an optic nerve fiber, but in a different location may produce no responses at all in this particular fiber. The distribution of sensitivity over the receptive field of a fiber may be determined by systematic exploration with a small spot of light, noting the responses elicited at various locations, and charting the boundaries of the region over which the spot is effective, at different intensities.

In figure 1 are given two examples. Figure 1a was obtained with a fiber whose responses consisted of a burst of impulses when the light was turned on, and another burst upon turning it off.[2] At the highest intensity ($\log I = 0.0$) the exploring spot (50 μ in diameter) would elicit responses if located anywhere within the outermost closed curve. The

[1] Although sharply focussed, such a spot of light on the retina is always surrounded by a faint halo of scattered light. This is due chiefly to Tyndall scattering in the layers of the retina overlying the rods and cones (diffraction, and reflection and scatter from the surfaces of the optical system contribute only a small amount). The relative intensity of this halo has been estimated by direct observation in several fresh preparations. A piece of gelatin neutral-tint filter was placed in the eye-piece of the dissecting microscope, just covering the image of the spot of light on the retina. With a large spot of light (1 mm. square), filters of densities between 2.0 and 3.0 were necessary to reduce the intensity of the spot, seen through the filter, to match approximately the intensity of the halo of scattered light, seen over the edge of the filter. Thus in nearly all cases the intensity of the halo, within a few microns of the edge of the spot, is 1 per cent or less of the spot intensity, and falls off rapidly with increasing distance from the edge of the spot.

[2] It has been shown previously that different optic nerve fibers of the vertebrate eye give different kinds of discharges in response to illumination of the retina. In some of the fibers impulses are discharged steadily as long as the light shines; others give only a brief burst of impulses when the light is turned on, and again when it is turned off; still others respond only to turning the light off. The general characteristics of the receptive fields of different fibers, however, are essentially the same, regardless of their type of response.

dots mark locations at which the spot could just elicit a response, at this intensity. For such locations on the boundary, both the "on" and the "off" bursts consisted of only one or two impulses, but locations inside the boundary gave rise to stronger discharges, and when the spot was located in the center of the region, vigorous bursts were obtained. At a lower intensity (1/100 of the former: log I = −2.0) responses could be obtained only when the spot was located within the much smaller region enclosed by the innermost curve, and at this intensity the discharges

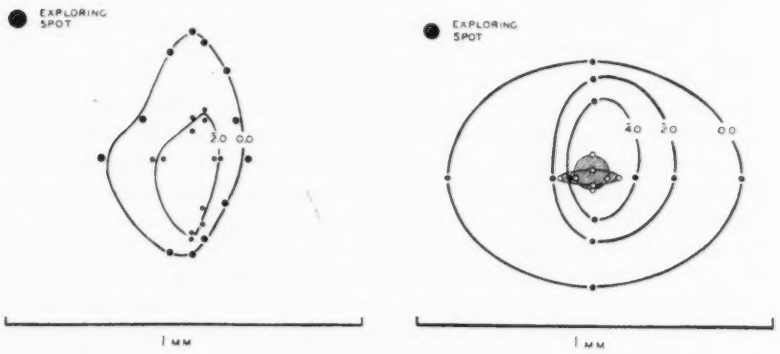

Fig. 1. Charts of the retinal regions supplying single optic nerve fibers (eye of the frog). a. Determination of the contours of the receptive field of a fiber at two levels of intensity of exploring spot. Dots mark positions at which exploring spot (50 μ diameter) would just elicit discharges of impulses, at the intensity whose logarithm is given on the respective curve (unit intensity = 2.10^4 meter candles). No responses at log I = −3.0, for any location of exploring spot. This fiber responded only at "on" and "off." b. Contours (determined by four points on perpendicular diameters) of receptive field of a fiber, at three levels of intensity (value of log I given on respective contours). In this fiber steady illumination (log I = 0.0 and −2.0) produced a maintained discharge of impulses for locations of exploring spot within central shaded area; elsewhere discharge subsided in 1–2 seconds. No maintained discharge in response to intensities less than log I = −2.0; no responses at all to an intensity log I = −4.6.

were very weak even when the spot was located in the center of the region. At a tenth of this intensity, log I = −3.0, no responses could be obtained for any location of the exploring spot whatever.

Figure 1b is a chart of the receptive field of another fiber, which in this case was capable of a steady discharge of impulses, maintained as long as illumination lasted. As in the previous experiment, the brighter the exploring spot, the larger was the region over which it would elicit responses, and, at any given intensity, the responses were stronger the more nearly central the location of the exploring spot. Indeed, it was

only for locations in the very center (cross-hatched region in fig. 1b) that the discharge would be maintained throughout an indefinitely long period of illumination. Elsewhere it would subside and finally stop in a second or two (cf. Hartline, loc. cit., fig. 6). At the lowest intensity represented in the figure (log I = −4.0) no maintained discharge could be obtained at all; the responses consisted of only a few impulses, and at $\frac{1}{4}$ of this intensity (log I = −4.6) no responses whatever could be elicited.

These experiments show that the sensitivity to light, referred to a particular optic nerve fiber, is not uniform over the fiber's receptive field. The central portion of the receptive field has a lower threshold and, at intensities above threshold, gives rise to stronger responses than the outlying areas. The sensitivity is thus maximal in the center, and falls off steadily with increasing distance from this center, to become inappreciable outside an area approximately one millimeter in diameter. Charts such as those of figure 1 are contour maps of this distribution of sensitivity. The faint halo of scattered light surrounding the exploring spot is a source of error in the construction of these charts. However, at relatively low intensities (100 or even 1000 times the minimum threshold) this scattered light is of little consequence, and a map obtained at these intensities must closely approximate the actual distribution of sensitivity over the receptive field of the fiber under observation.

Factors other than the absolute intensity of the exploring spot affect the extent of the region from which responses in a given fiber can be elicited. If the exploring spot is made smaller, its intensity must be increased if it is to be effective over as large an area. But with this smaller spot the threshold measured in the most sensitive central region is correspondingly increased. It is the intensity relative to this minimum threshold which is significant in charting the distribution of sensitivity. Similarly, if the retina is not completely dark adapted, its level of sensitivity is decreased, and for a particular fiber the thresholds in the center and on all the contours of its receptive field are increased proportionately. Receptive fields of different fibers must likewise be compared with due regard to their minimum thresholds, which may differ considerably.

The vertebrate retina responds vigorously to small, sudden movements of the retinal image (Ishihara, 1904; Adrian and Matthews, 1927). This may be observed in the responses of single optic nerve fibers, and is helpful in determining the distribution of sensitivity in their receptive fields. Figure 2 shows records of the discharge in a fiber responding at "on" and "off." Although no impulses were discharged while the spot of light was shining steadily, a slight movement of it, of only a few microns in any direction, produced a short burst of impulses. Responses to movement are stronger, within limits, the larger and more intense the moving spot, and the greater and the more rapid its displacement. Responses to a

slight movement of a spot of light of given size and intensity can be elicited anywhere within the region over which this spot is effective in producing discharges when it is turned on or off. They are weak when the spot is near the boundary of this region, and stronger the more nearly central its location in the receptive field. Figure 3 shows the contour

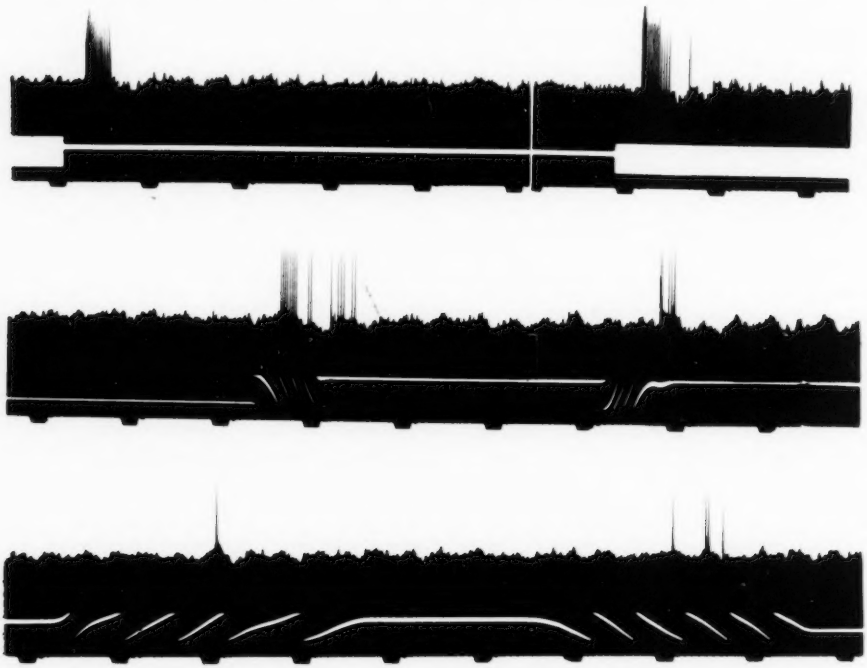

Fig. 2. Oscillograms of action potentials in a single optic nerve fiber (frog), showing responses to slight movements of small spot of light (50 μ diameter) on the retina. Fiber responded only at "on" and "off"; no discharge during steady illumination if stimulus spot was stationary (upper record; signal marking period of illumination blackens the white strip above time marker). Slight movements of stimulus spot elicited short bursts of impulses (middle and lower records). Movements of spot on retina are signalled by narrow white lines appearing above time marker; these are shadows of spokes attached to head of micrometer screw controlling position of stimulus spot. Each spoke corresponds to 7 μ on the retina. Time in $\frac{1}{5}$ second.

within which a spot of light 50 μ in diameter, about 100 times the minimum threshold, produced responses in a fiber responding to "off" only. The arrows show the limits, on two diameters, between which slight movements of this spot (ca. 20 μ in ca. 0.05 sec.) would elicit bursts of impulses. Outside of these limits no responses to movement could be

obtained, no matter how great or how rapid the displacements. It is characteristic of a fiber which responds only to "off" that it also responds only to movements of the spot away from the center of its receptive field.

Bursts of impulses are also elicited in response to movement of a shadow on the uniformly illuminated retina. A slight, sudden movement of a narrow band of shadow produces responses if it falls across the receptive field of the fiber under observation, and these responses can be elicited over a region many times wider than the shadow itself. To show this, all diaphragms were removed from the optical system, and a fine wire

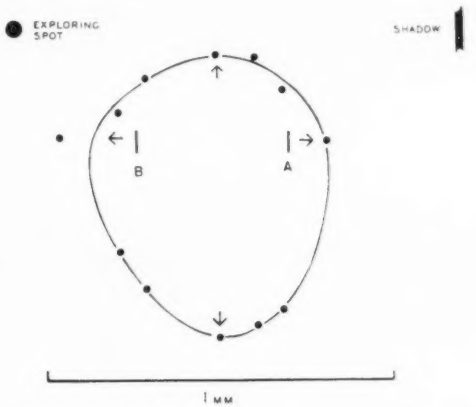

Fig. 3. Chart of the receptive field of an optic nerve fiber (frog), showing limits within which responses were elicited by movements of an illuminated spot, and of a narrow band of shadow. Dots mark locations at which exploring spot produced responses when turned off (fiber responded only to "off"). Spot 50 μ in diameter, intensity 100 times minimum threshold. Arrows mark the limits (on two diameters) between which slight movements of illuminated spot elicited bursts of impulses. With large area of retina illuminated (4 mm. diameter) a band of shadow 20 μ wide produced discharges of impulses when moved slightly, if it crossed the receptive field within the limits marked by the vertical lines *A* and *B*. Shadow extended across entire illuminated area, in direction lengthwise of page; movements were crosswise. See figure 4 for records of responses to moving shadow.

was stretched across the beam. This yielded a circular patch of light on the retina, 4 mm. in diameter, across which was a band of shadow 20 μ wide. In the experiment of figure 3 the limits within which slight movements of this shadow produced responses are indicated by the vertical lines, A and B. If the shadow was near either of these limits the responses to its movement were weak, as shown in the upper and the lower records of figure 4, while if it fell across the center of the sensitive region the same amount of displacement elicited stronger bursts of impulses (middle record of fig. 4). Responses to movement of a shadow are elicited regardless of

the direction of the motion, both in the fibers responding to "on" and "off" and in those responding to "off" only.

From these experiments it is evident that the receptive field of an optic nerve fiber from the peripheral retina covers an area much greater than

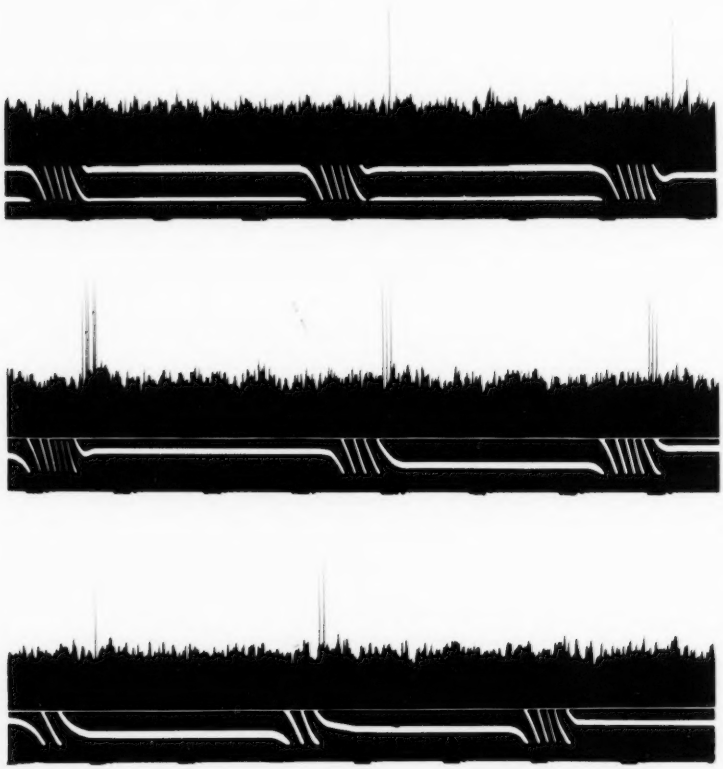

Fig. 4. Records of the impulses discharged in an optic nerve fiber in response to movement of a shadow on the retina. Experiment of figure 3. Narrow band of shadow, on uniformly illuminated retina, was moved from right to left (chart of fig. 3) in a series of short, quick jerks. First response (upper record) occurred at position A in figure 3; responses elicited to every succeeding movement until position B was reached (lower record shows last response). Responses were strongest midway between (middle record). Signal of movement as in figure 2. Time in $\frac{1}{5}$ second.

that occupied by a single receptor cell. The receptor elements are small, even compared to the exploring spot used in these experiments; consequently, if illumination of but one rod or cone gave rise to the responses in a given optic nerve fiber, charts such as figure 1 would be faithful

representations of the distribution of light associated with the exploring spot. Direct observation of this spot on the retina showed that it was small and sharply focussed, with a halo of scattered light at most only $\frac{1}{100}$ as intense as the spot itself. Yet this spot, at intensities only 4 to 10 times the minimum thresholds for the various fibers, elicited responses over regions many times its own diameter. The observed distributions of sensitivity, with broad maxima several tenths of a millimeter in diameter, in no way resembled the minute exploring spot, only 50 μ in diameter, as they would have if only a single receptor cell had been responsible for the excitation of each optic nerve fiber. Likewise, high sensitivity to slight movement of the spot was not found to be restricted to regions as small as the stimulus spot itself. Finally, the use of the narrow band of shadow upon the uniformly illuminated retina definitely rules out possible effects of scattered light. The sensitivity to slight movements of this shadow, over a region many times its width, offers conclusive proof that many receptor cells are concerned in the excitation of a single optic nerve fiber.

A retinal ganglion cell, therefore, can receive excitatory influences over many convergent pathways; its axon is the final common path for nervous activity originating in many sensory elements. This, of course, is in keeping with the known anatomical organization of the vertebrate retina. It furnishes the functional basis for the spatial effects in the vertebrate retina, observed in experiments on the whole optic nerve by Adrian and Matthews (1927, 1928). They found that the latency of the optic discharge was shorter the greater the area of the retina illuminated, and attributed this to summation of the excitatory effects due to activity in convergent retinal pathways. It is worthy of note that this spatial summation was limited to retinal distances of approximately 1 mm., which is the order of magnitude of the diameter of the receptive fields of the single optic nerve fibers. Moreover, the spatial effects were smaller the greater the retinal distances, in keeping with the diminished effectiveness of the outlying regions of the receptive fields. This diminished effectiveness may be ascribed to a smaller number of receptor elements in a unit area that are in connection with a given retinal ganglion cell, or to a less effective transfer of nervous activity over the longer and less direct pathways from the margins of the receptive field.

The receptive fields of different fibers may overlap considerably (Hartline, loc. cit.). Consequently, illumination of a single point on the retina can produce activity in many different fibers, and illumination of two discrete points may produce activity in many fibers in common. It is for this reason that fine detail cannot be resolved by the peripheral retina. From the standpoint of visual function, it is necessary to consider the distribution of activity among the different fibers of the optic nerve,

elicited by illumination of a particular small element of area on the retina.

A bundle containing a number of active optic nerve fibers may be used to sample this distribution. If not too many fibers are present, it is possible to distinguish the activity in the different ones by means of the loud speaker and the cathode ray oscilloscope. When the responses in such a bundle are tested it is at once apparent that many fibers are excited by a small spot of light (50 μ in diameter), even at intensities close to threshold. Certain of the fibers respond vigorously to the light; these are the ones whose receptive fields are centered close to the stimulus spot. Others give only feeble responses; these either have higher thresholds, or are fibers whose receptive fields are centered at some distance from the stimulus spot, which consequently falls in the less sensitive peripheries of their fields. When the spot of light is tested in a slightly different location on the retina, it is strikingly evident that the composition of the response is changed. Fibers which had been active cease responding, new fibers come into play, fibers which had given strong responses give weak ones, and some of those which had only given slight discharges dominate the response.

It is evident that illumination of a given element of area on the retina results in a specific pattern of activity in a specific group of optic nerve fibers. The particular fibers involved, and the distribution of activity among them, are characteristic of the location on the retina of the particular element of area illuminated. Corresponding to different points on the retina are different patterns of nerve activity; even two closely adjacent points do not produce quite the same distribution of activity, although they may excite many fibers in common. The more widely two illuminated spots are separated the fewer fibers will be involved in common, but it is reasonable to suppose that it is only necessary to have two recognizable maxima of activity in order to resolve the separate spots. It is this spatial specificity of groups of optic nerve fibers, and of the distribution of activity among them, that furnishes the basis for distinguishing the form of the retinal image.

SUMMARY

The region of the retina which must receive illumination in order to elicit a discharge of impulses in a particular optic nerve fiber is termed the receptive field of that fiber. Characteristics of the receptive fields of individual optic nerve fibers from the peripheral retinas of cold-blooded vertebrates (frog, alligator) have been investigated by recording the action potentials in single fibers in response to illuminating various parts of the retina. In several experiments the distribution of sensitivity over the receptive field of a particular fiber has been determined by systematic

exploration of the retina with a small spot of light, noting the responses elicited in the fiber at various locations, and charting the boundaries of the region over which the spot is effective, at various intensities.

The sensitivity to light, referred to a particular optic nerve fiber, is maximal over the central portion of the fiber's receptive field, where the threshold is lower than in the outlying areas, and where intensities above threshold give rise to the strongest responses. The sensitivity is less the greater the distance from this central region, and is usually inappreciable outside an area about one millimeter in diameter. Contour maps of the distribution of sensitivity are given for two examples.

Single optic nerve fibers (of the types responding to "on" and "off," and to "off" only) respond to sudden, slight movements of an illuminated spot, or of a band of shadow on the uniformly illuminated retina, if the moving spot or shadow falls within the receptive field of the fiber. Movements of only a few micra of a small spot or a narrow shadow can elicit responses in a particular optic nerve fiber over a retinal region several tenths of a millimeter in diameter—many times the width of the spot or shadow.

These experiments prove that the receptive field of an optic nerve fiber from the peripheral retina covers an area much greater than that occupied by a single rod or cone. A retinal ganglion cell, therefore, can receive excitatory influences over many convergent pathways; its axon is the final common path for nervous activity originating in many sensory elements. This finding furnishes the functional basis for the spatial effects observed in the peripheral vertebrate retina.

Action potentials recorded from small bundles containing many active optic nerve fibers show that a single small spot of light excites many fibers: the receptive fields of different fibers overlap considerably. The particular fibers activated, and the distribution of activity among them, is characteristic of the location on the retina of the particular element of area illuminated. This spatial specificity of groups of optic nerve fibers, and of their patterns of activity, furnishes the basis for distinguishing the form of the retinal image.

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THE EFFECTS OF SPATIAL SUMMATION IN THE RETINA ON THE EXCITATION OF THE FIBERS OF THE OPTIC NERVE[1]

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In a previous paper (Hartline, 1940) it was shown that a ganglion cell in the peripheral retina of the vertebrate eye is excited by activity in many convergent pathways, from sensory elements distributed over a receptive field covering approximately a square millimeter of retinal area. Illumination of any portion of the receptive field of a retinal ganglion cell will accordingly produce a discharge of impulses in its axon, the strength of the response to illumination of a fixed retinal area usually being greater the higher the intensity of the stimulating light. The present paper will show that the discharge of impulses in a single optic nerve fiber also depends upon the size of the illuminated area. The excitation of a ganglion cell is therefore controlled by the number of active pathways which converge upon it, as well as by the degree of activity in the individual pathways.

Spatial summation in the vertebrate retina has previously been demonstrated by Adrian and Matthews (1927–1928). They showed that the latency of the discharge of impulses in the whole optic nerve of the eel was shorter the larger the area of the retina illuminated, and the latency of the response to four spots of light was shorter than the shortest latency obtained with any of the spots singly. This summation was enhanced by the application of strychnine, indicating that it depended upon the nervous interconnections within the retina. The study of the activity in single optic nerve fibers has now furnished more direct evidence for the convergence of excitatory effects within the retina; the present paper is concerned with the extension of this study to an analysis of spatial summation, in terms of the activity of the individual units of the retina.

METHOD. The method for studying the activity of single optic nerve fibers in the retinas of cold-blooded vertebrates, and for determining the location and extent of their receptive fields has been described in previous papers (Hartline, 1938, 1940). In the present experiments the eyes from

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large frogs (R. catesbiana) were used. None of the receptive fields of the fibers studied lay within or near the *area acuta* of the retina; the properties here reported are those of the peripheral retina.

The apparatus for illuminating the retina has likewise been described previously. It provided a beam of light which could be directed upon any part of the exposed retina, more than large enough to cover the region under investigation. The illumination was restricted to any desired area within this beam by means of diaphragms, with apertures of suitable size and shape imaged on the retina. Sharpness of focus was assured, in every experiment, by direct observation of the patterns of light on the retina by means of a dissecting microscope (×32). The diaphragms were readily interchangeable, and slipped into place against mechanical stops in a holder. The accuracy and reproducibility of their alignment in the beam was checked by exposing photographic plates in the place of the retina. Fine adjustments on the diaphragm holder enabled it to be shifted slightly, within the limits of the beam, so that the patterns of illumination could be accurately centered upon the receptive field of the fiber under observation.

Results. In figure 1 are shown oscillograms of the amplified action potentials in a single optic nerve fiber, obtained in response to illumination of the retina with patches of light of various sizes. The areas illuminated, which were circular in shape, had been carefully centered upon the most sensitive portion of the fiber's receptive field, and fell well within its limits. The larger the area of the stimulus patch, the shorter was the latency of response, and, for moderate degrees of stimulation, the higher was the frequency and the greater the number of impulses in the discharge. The fiber used in this experiment was one responding with a burst of impulses at the onset of illumination, and again when the light was turned off (no discharge during steady illumination). Fibers giving other types of response (cf. Hartline, 1938) show a similar dependence of the discharge upon the area illuminated.

Varying the area of the retina illuminated by a fixed intensity thus affects the response in a single optic nerve fiber: this effect, moreover, is exactly similar to that obtained by varying the intensity of illumination upon a fixed retinal area (cf. Hartline, 1938). To permit a comparison, two series of records are shown in figure 1, obtained at two different intensities of illumination. The responses in the right hand column were obtained with an intensity ten times that used in the left. It is to be noted that responses at the higher intensity are comparable to those obtained with areas approximately ten times larger, at the lower intensity. Only the total luminous flux falling upon the retina (area × intensity) is of significance in determining the response of the ganglion cell. This rule has been found to hold, except for very strong stimulation, to within the

limits of accuracy of this method. It applies only to illumination falling well within the receptive field of the fiber under observation.

A simple demonstration of this reciprocal relation between the area and intensity necessary to produce a constant effect in an optic nerve fiber is furnished by the determination of the threshold intensity, $I_{thresh.}$,

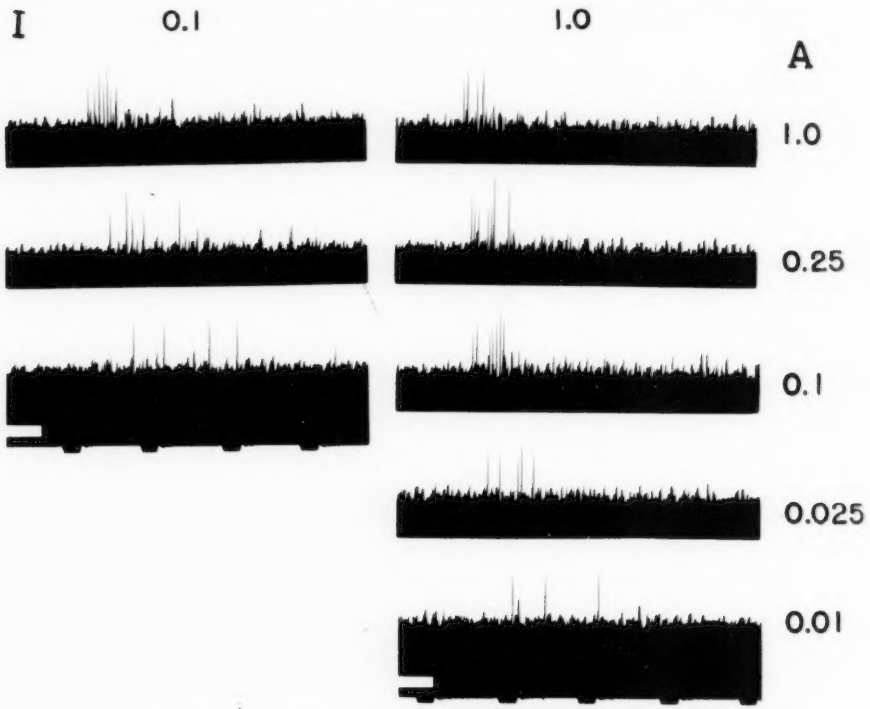

Fig. 1. Oscillograms of action potentials in a single optic nerve fiber from a frog's retina, showing effect of size of stimulus patch upon the discharge of impulses. Retina illuminated with circular patches of light, centered on receptive field of the fiber; relative areas (A) given on right (A = 1 corresponds to 0.006 mm.^{2}). For the responses in the left hand column the intensity of illumination was $\frac{1}{10}$ that used for the right hand column. (I = 1 equivalent to 3.10^3 meter candles). Fiber was one responding with bursts of impulses at "on" and at "off" with no impulses discharged during steady illumination. Only "on" burst shown here. Signal of illumination blackens white line above time marker (only shown in bottom records). Time in $\frac{1}{5}$ second.

for various areas, A, of retinal illumination, plotted in figure 2. The line through the experimental points has a slope of -1, representing, on this double logarithmic plot, the relation

$$A \cdot I_{thresh.} = \text{constant.}$$

This relation was demonstrated by Adrian and Matthews (1927-1928) in the optic discharge of the eel's eye; the present experiments show it to be a property of the individual retinal ganglion cells. Its limitation to retinal distances less than 1 mm., as reported by Adrian and Matthews, is due to the fact that the diameter of the receptive field of a ganglion cell is, on the average, of this order of magnitude.

Measurements of the reciprocal of the latent period and of the initial frequency of the discharge of impulses (in the same fiber whose responses are shown in fig. 1) are plotted, in figure 3, as functions of the area of illumination, for various levels of intensity. For moderate degrees of stimulation, these measures of the response increase steadily and approximately linearly with the logarithm of the area illuminated. Curves ob-

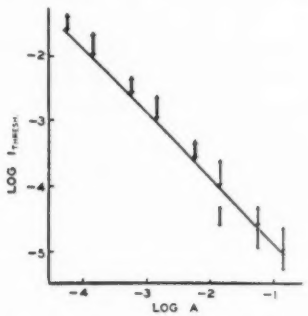

Fig. 2. Relation between area of retina illuminated (A) and threshold intensity, $I_{thresh.}$ for stimulation of a single optic nerve fiber. For each arrow, upper point gives lowest intensity which elicited one or two impulses; lower point gives highest intensity which failed to elicit any response (determinations made to nearest 0.3 or 0.4 log unit). Where duplicate determinations coincided, arrows are drawn heavier. Line drawn through points has slope of -1. (Log I = 0 equivalent to 3.10^5 meter candles; area in mm.^{2}.)

tained at different levels of intensity are separated, parallel to the axis of abscissae, by amounts roughly equal to the logarithms of the ratios of their intensities, in accordance with the reciprocity relation discussed above.

Figure 3 shows that the responses increase with increasing area only up to a certain point. Beyond this point the responses actually decrease with increasing size of stimulus area, although these areas are well within the limits of the receptive field of the fiber. It is furthermore to be noted that the higher the intensity the smaller is the area at which this decrease begins. This effect may also be seen in the right hand column of figure 1, where the response to the largest area contains fewer impulses than the response to the area one-fourth as large. A similar depressing effect on the response has been reported, when the intensity of retinal illumination

on a fixed retinal area is increased above an optimal value (Hartline, 1938). It is as though the ganglion cell can be "overloaded," and the fact that this can be accomplished by increasing the area of the retina illuminated, as well as by increasing the intensity of the light, serves to emphasize the principle that the final response of the ganglion cell is determined by the sum total of activity reaching it over many convergent pathways.

It has been pointed out previously that in these experiments the sensitivity to light of any point on the retina must be defined with respect to the particular optic nerve fiber under observation. The sensitivity, thus defined, is not uniform over the receptive field of a fiber; the outlying portions are less effective in producing responses than is the central region (Hartline, 1940). It is reasonable to suppose that the outlying portions of the receptive field also contribute less to the total summed excitation

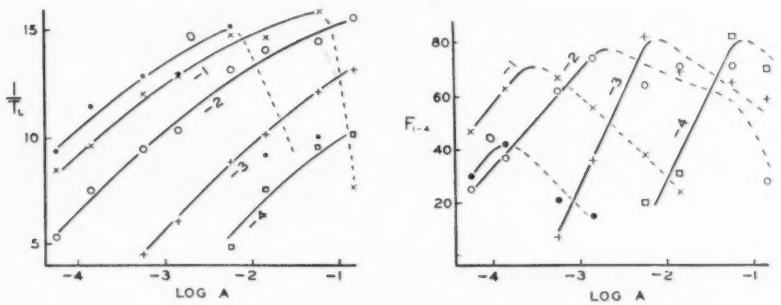

Fig. 3. Relation between area of retina illuminated (A) and response in a single optic nerve fiber, at five levels of intensity. (Measurements of the complete experiment from which the records shown in fig. 1 were selected.) a. Reciprocal of latent period in seconds, T_L, of "on" burst vs. log A (in mm.^{2}). The number on each curve gives the logarithm of the intensity of illumination for that curve (log I = 0 equivalent to 3.10^5 meter candles). b. Initial frequency of discharge of "on" burst (F_{1-4}; 1st 4 impulses) vs. log A. Numbers on curves give respective values of log I.

of the ganglion cell. To test this point, and to study the relative contributions from the component portions of an illuminated area under different conditions, the following series of experiments have been performed.

A square area, large enough to cover nearly all of the receptive field of a fiber under observation, was subdivided into 25 small squares by means of diaphragms with appropriate apertures. Each of these small areas could be illuminated separately and the response to it compared with the response to illumination of the entire area, or of areas comprising several of the small subdivisions.

The requirements for threshold excitation of a fiber responding at "on" and "off" (only the "on" response recorded) are given in figure 4. The minimal intensity necessary to produce a response was determined for each

small square illuminated alone, and also for areas covered by 4, 9 and 25 of these small squares, as indicated in the figure. The reciprocals of these threshold intensities are entered in the respective squares, so that the greater the number in a particular square the more effective was that

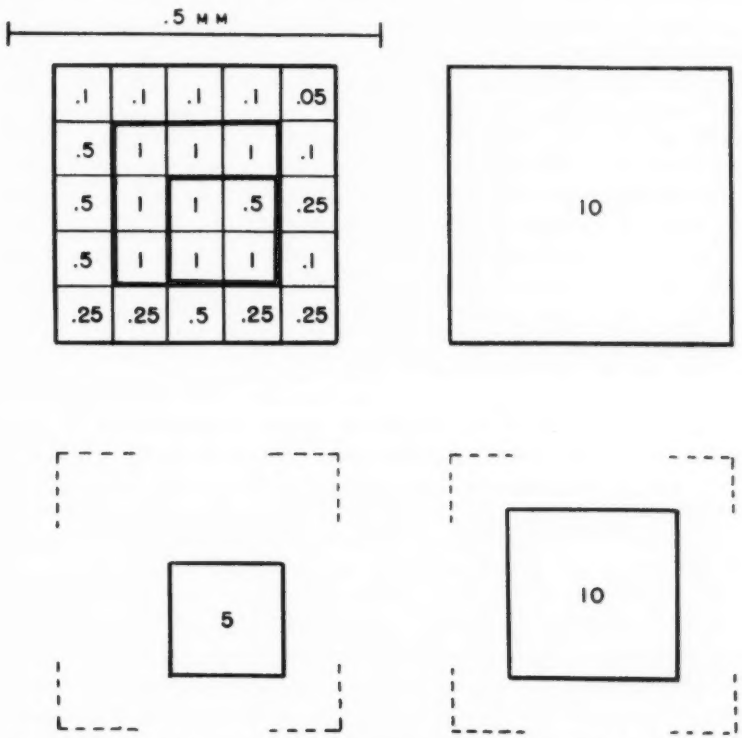

Fig. 4. Chart of the relative effectivness, in stimulating a single optic nerve fiber, of different portions of the fiber's receptive field. "Effectiveness" of a region of the retina defined as reciprocal of threshold intensity for that region. Upper left: numerical values of effectiveness of 25 subdivisions of large square area tested individually. (Comparative scale of retinal distance given above.) Threshold intensity of the most effective subdivisions set equal to 1 (equivalent to 8.10^{-3} meter candles) Lower left: effectiveness of area covering 4 of the central subdivisions (heaviest outline in upper left). Lower right: effectiveness of area covering the 9 central subdivisions (heavy outline in upper left). Upper right: effectiveness of entire large square. Fiber gave "on" and "off" bursts. "Threshold" taken as the lowest intensity (within 0.3 or 0.4 log unit) which would reliably produce an "on" burst of one or two impulses.

area in producing excitation of the ganglion cell. It is to be seen that the region of maximum sensitivity of the receptive field of this fiber was covered by eight of the nine central squares; the 16 border subdivisions

were all considerably less effective. When the larger area covered by four of the central squares was illuminated, the threshold intensity was one-fifth that of any of its subdivisions alone; when the still larger area covered by the nine central squares was exposed the threshold was still lower—only one-tenth of the threshold of the most sensitive subdivision. Thus, for the central portion of the receptive field, large illuminated areas were more effective in exciting the ganglion cell than any of their subdivisions. However, when the entire area covered by the 25 small squares was illuminated, the threshold intensity was not measurably lower than the threshold of the central region covered by only nine squares. Adding the 16 border subdivisions did not appreciably increase the effectiveness of the illumination, in this experiment. To judge from other experiments, the outlying portions of the receptive field do contribute somewhat to the total effect, and this might have been observed in the present experiment, had the thresholds been determined more closely. Nevertheless, the inclusion of less sensitive regions of the receptive field contributes correspondingly little to the summed effect; illumination of areas entirely outside the receptive field contributes nothing at all to the excitation of the ganglion cell.

Spatial summation in the vertebrate retina is thus limited to the receptive field of the retinal ganglion cell, and its effects are most readily observable in the more sensitive central portion of that field. A series of experiments has been performed, designed to analyze the contributions from component subdivisions of an illuminated area, which in every case lay well within the receptive field of the fiber under observation.

The experiment of figure 4, just cited, furnishes evidence of the summation of subliminal excitation. Thus illumination of any single square at an intensity $1/I = 10$ failed to produce a response, yet this illumination must have produced some degree of activity in the pathways converging upon the ganglion cell, for when the nine central squares were illuminated together, at this intensity, impulses were discharged in the optic nerve fiber. Another example is furnished by an experiment on a fiber responding only to the cessation of illumination. At a suitable intensity, illumination of any one of four small squares singly produced no responses, but when all four were illuminated together "off" responses were regularly elicited, consisting of at least 7 impulses, at frequencies of 45 to 60 per second. Evidently, weak light can produce effects in the individual subdivisions of an area which are subliminal when they act alone, but which sum to reach the threshold of the ganglion cell when all act together. Since the activity in the retinal pathways presumably involves nerve impulses, we must conclude that more than one impulse must reach the retinal ganglion cell in order to excite a response in its axon.

The experiment of figure 1 shows that spatial summation not only affects

the threshold intensity to which a ganglion cell will respond, but also determines the magnitude of response at intensities above threshold. By testing different subdivisions of an area separately it can be shown, first, that the responses to illumination of a given area may be augmented by subliminal excitation from adjacent regions of the receptive field, and second, that illumination strong enough to elicit responses from each single

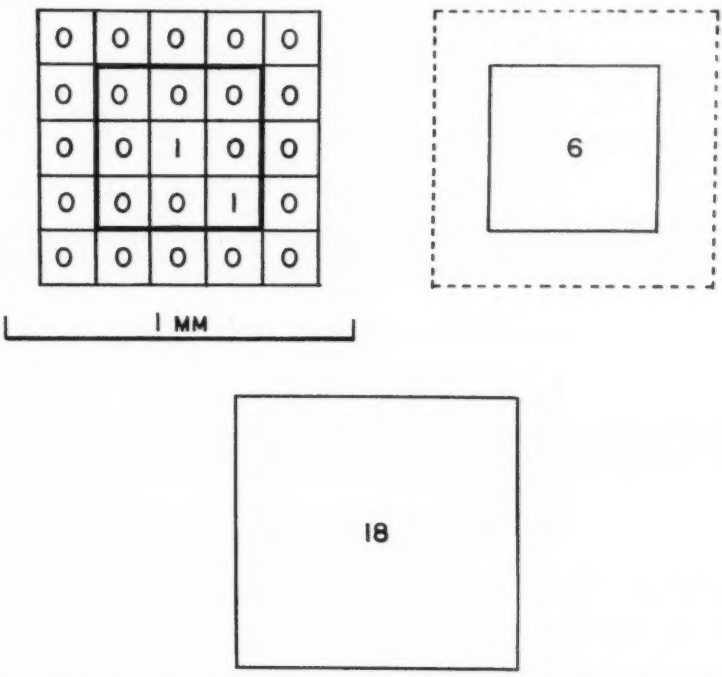

Fig. 5. Chart of the responses of a single optic nerve fiber to illumination of different portions of its receptive field, at a fixed intensity (2.10^{-3} meter candles). Upper left: number of impulses in response to each of 25 subdivisions of large square, tested individually (comparative scale given below). Upper right: response to illumination of area covered by 9 central subdivisions (heavy outline in upper left). Below: response to illumination of entire square. Fiber responded only to "off." Duration of exposure for each test *ca.* 5 sec.

subdivision of an area produces still greater excitation when the total area is exposed.

In an experiment (fig. 5) on a fiber responding only to cessation of illumination, only two of the central squares, out of the 25, would elicit a response (one impulse) when illuminated singly. However, when the area covered by the nine central squares was exposed, at this same intensity,

responses of 6 to 10 impulses, at average frequencies of 10 to 20 per second, were elicited. And when the 16 border subdivisions were added, the response increased to 18 impulses, at 53 per second, although none of these border squares alone could produce any response at this intensity. While

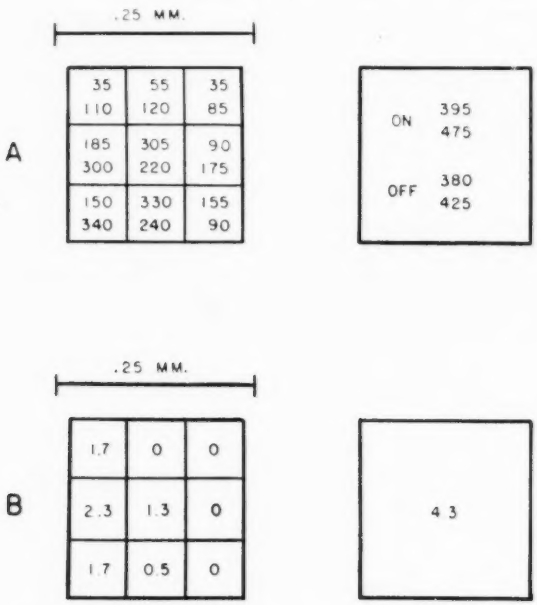

Fig. 6. a. Chart of the responses of a single optic nerve fiber (responding at "on" and at "off") to illumination of different portions of its receptive field, at a fixed intensity (0.3 meter candles). Left: frequencies of discharge (1st 6 impulses) of "on" and "off" bursts (upper and lower numbers, respectively) for each of 9 small squares tested individually (scale of distance given above). Right: frequencies of discharge of the "on" and "off" bursts (upper and lower pairs of numbers, respectively) in response to illumination of entire area covered by the 9 small squares. Upper member of each pair of numbers gives value obtained before testing the small squares, lower member the value afterwards. b. Chart of the frequencies of maintained discharge (13th to 15th second of continuous illumination) of single optic nerve fiber, in response to illumination of each of 9 small squares (left) compared with response to illumination of entire area covered by these squares (right). Scale given above. Intensity 300 meter candles.

it has been shown that border subdivisions contribute less to the summed effect of the illumination than do the more central ones, this experiment shows that their contribution nevertheless may be quite appreciable. This is especially true at low levels of excitation, where a slight increase in the stimulus usually causes a considerable increase in the response.

At an intensity moderately above threshold, the response to illumination of a large area is greater than the greatest response to illumination of any subdivision of this area at this same intensity. Illumination of nine small squares individually at an intensity above threshold resulted in the responses tabulated in figure 6a. When the entire area covered by these nine squares was exposed, at this same intensity, the frequency of the discharge was greater than in the responses of even the most effective subdivision illuminated alone. With fibers of this kind, responding to a change in illumination, both the "on" and the "off" bursts show the effects of spatial summation. A similar result, with a fiber whose discharge was maintained during steady illumination, is shown in figures 6b and 7. The frequency of the steady discharge resulting from illumination of each

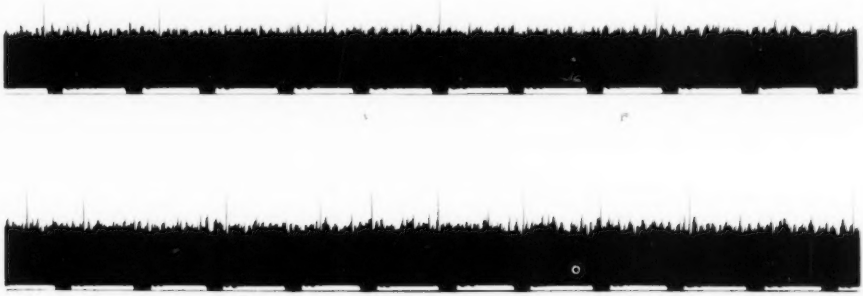

Fig. 7. Records of the maintained discharge of impulses in a single optic nerve fiber, showing effects of spatial summation. Top: response to illumination of most effective one of 9 subdivisions of an area of the retina (small square labelled 2.3 in fig. 6, b). Bottom: response to illumination of entire area covered by the 9 subdivisions (labelled 4.3 in fig. 6, b). Records include the 13th to 15th seconds of steady illumination. Intensity 300 meter candles. Time marked in $\frac{1}{4}$ second.

of the subdivisions singly is given in the respective square in figure 6b. When the entire area was illuminated, the frequency of the resulting discharge exceeded the highest frequency obtained from any of the small squares alone. Figure 7 shows the records of the responses to illuminating the entire area and to illuminating its most effective subdivision at the same intensity.

As noted previously, excitation above an optimal limit results in diminished responses in an optic nerve fiber. Thus it can happen that the response to the total area is actually less than that to any of its component subdivisions. The fiber, cited above, whose "off" responses illustrated the summation of subliminal effects from four subdivisions of an area, gave the following responses when tested at an intensity 100 times higher. The individual squares, illuminated singly, gave "off" bursts having

initial frequencies of 265, 230, 205 and 195 impulses per second. In response to illuminating the whole area covered by these four squares, at the same intensity, the initial frequency of impulses in the burst was only 175. That this diminished response was due to the excessively high total excitation was shown by reducing the intensity of the light to $\frac{1}{4}$ its previous value; illumination of the whole area then gave a response whose initial frequency was 240 impulses per second. Summation of excitation due to activity in convergent pathways takes place over the entire range of the response of the retinal ganglion cell.

Spatial summation can take place, of course, only where there is convergence of the effects of stimulation. In the more simple eye of *Limulus*, there is no convergence, and the response in a given optic nerve fiber depends only upon the illumination of the sensory cell giving rise to that fiber. Illumination of adjacent areas of the eye has no effect upon this response (Graham, 1932). But where there is convergence there need not be summation; the response in the final common path might be determined solely by the most strongly excited component. This is not so in the vertebrate retina, as was originally evident from the studies of Adrian and Matthews. The present experimental study furnishes direct evidence that the excitation of a single retinal ganglion cell is determined by the summated effects of activity in the pathways converging upon it.

SUMMARY

A study has been made of the action potentials of single optic nerve fibers of the frog's retina, in response to illuminating areas of the retina of various sizes. In these experiments the fibers used were from the peripheral retina, where many receptor elements are connected with each retinal ganglion cell.

The discharge of impulses in a single optic nerve fiber is stronger the larger the area of the retina illuminated, within the limits of the fiber's receptive field. Except for very strong illumination, the responses have a shorter latency and a higher frequency the greater the number of receptors illuminated. The threshold intensity is also lower the larger the area of the stimulating patch of light.

Varying the area of the retina illuminated by a fixed intensity affects the discharge of impulses in a single optic nerve fiber in the same way as varying the intensity of illumination of a fixed area. For threshold excitation and for levels of response above threshold, only the total quantity of light (A·I) determines the response, provided the illumination is confined to the central portion of the fiber's receptive field.

Excitation of a retinal ganglion cell above an optimal limit results in diminished responses in its optic nerve fiber: this effect can be produced by increasing either the intensity or the area of the retinal illumination.

The discharge of impulses in response to illumination of a given area within the receptive field of an optic nerve fiber has been compared with the responses to illumination of subdivisions of this same area. 1. Illumination of the less effective subdivisions in the margins of the receptive field contributes correspondingly little to the summed effect upon the ganglion cell. Illumination of areas entirely outside the receptive field has no effect upon the discharge of impulses. 2. Subliminal effects from the subdivisions of an area can sum to reach the threshold of the ganglion cell when all the subdivisions are illuminated together. From this it is concluded that more than one nerve impulse must reach the retinal ganglion cell, over the pathways converging upon it, in order to excite a discharge in its optic nerve fiber. 3. The discharge of impulses in response to illumination of a given area is stronger than the strongest response from any subdivision of this area, illuminated at the same intensity. This is true provided the ganglion cell is not stimulated too strongly; at very high levels of excitation the response to illumination of the entire area is diminished.

An optic nerve fiber is the final common path for nervous activity originating in many receptor elements of the retina; excitation due to the activity in the retinal pathways converging upon a single ganglion cell summates to determine the response in its optic nerve fiber.

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TEMPERATURE REGULATION IN CHRONIC CERVICAL CATS[1]

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A number of investigators have studied the effects of section of the spinal cord at the cervical level on temperature regulation and have reported conflicting results. The greatest damage to temperature regulation following such an operation was reported by Freund and Strasmann (1912). They found that cervical rabbits, i.e., rabbits with the spinal cord severed in the cervical region, when subjected to very slight changes in environmental temperature showed such marked changes in rectal temperature that the animals could be considered poikilothermic. None of their animals survived more than 11 days and the nutritive state was poor. Sherrington (1924) did not find such great damage to temperature regulation in cervical dogs, although he does state that it was necessary to keep them in a room heated to about 80°F. in order for them to maintain normal body temperatures. His animals were able to pant but they were unable to prevent their temperatures from rising under conditions where normals showed little or no change in rectal temperature. In these dogs he noted an absence of peripheral vasomotor changes and of erection of hair and in addition he noted that shivering only occurred in those regions whose innervation came from above the level of the cord section. Although the majority of the tests which he performed were quite drastic they clearly demonstrated the inadequacy of temperature regulation in cervical dogs as compared to normals. If one examines closely the work of Issekutz et al. (1937) it will be seen that only when his cervical cats were kept in rooms at about 80° were their body temperatures normal.[2] In contrast to the above, other workers have stated that cervical animals possess quite efficient temperature regulation. Popoff (1934) studied dogs in which the spinal cord was destroyed below C_5C_6 and in which the vagi had been sectioned at the level of the thyroid cartilage. He stated that such animals had the same temperature as normal dogs when kept at room temperatures of 50°, and that he had observed vaso-

[1] Aided by a grant from the Rockefeller Foundation.

[2] In a series of 474 observations of morning rectal temperature on 32 unselected cats at varying room temperatures of about 65 to 85° the mean was 101.37° with a standard deviation of 0.79° (Clark, unpublished observations).

constriction in response to cold and vasodilatation in response to heat. Quite unexplainably he also stated that "das Fell der Hunder bedeckte sich manchmal mit 'Schweisstropfen.' " Except for the latter, somewhat similar results have been obtained by Hermann, Morin and Crier (1938), Hermann, Morin and Galy (1938) and Morin (1938). Thauer (1935) states that after an initial period of thermolability (6–8 days) cervical rabbits can be kept at room temperatures of 65 to 70° and later can withstand room temperatures of 40° without the body temperature falling. He further reported that such animals could withstand overheating as well as normals and that changes in the size of ear vessels occurred in response to changes in environmental temperature. The longest survival which he reported was 70 days.

Because of this difference in results it seemed desirable to reinvestigate the ability of cervical animals to regulate their body temperature. Cats were utilized because considerable information has been accumulated on temperature regulation in normal cats (Clark, Magoun and Ranson, 1939). It was decided to prepare two series of animals in order to determine 1, the amount of recovery in ability to withstand chilling, and 2, the effect of long continued exposure to temperatures, sufficiently low to produce slightly subnormal temperatures, on the ability to withstand chilling. In each cat a laminectomy was performed under ether anesthesia, the cord cut in the lower cervical region and a small segment (1–2 mm.) removed. At autopsy the level of the transection was determined. In the second series the portion of the cord containing the scar was removed, fixed in formalin, embedded in nitrocellulose and stained by the Van Gieson method.

It was found that the care of high spinal animals was more time consuming than difficult. Catheterization was usually necessary for the first 3 days or so, but after that the urine could be pressed out. Occasionally enemas were necessary. The animals were supported on tightly stretched fish net and frequently turned to prevent the development of pressure sores. By careful cleaning perineal ulcers were avoided. The diet consisted of 50 grams of ground beef hearts night and morning. At very infrequent intervals liver or "Pard" was substituted. Most of the cats gained in weight and at autopsy had a thick layer of subcutaneous fat.

First series. At first it was necessary to keep the animals in an incubator set at 90°, but after about a week they were placed in a room with an even temperature of approximately 82°. At weekly intervals throughout the 2½ months that this group was allowed to survive, their ability to react against cold was tested by placing them for from 1 to 5 hours in a room at about 68°. In most of the experiments made to determine the ability of cervical cats to withstand chilling a room designed for paraffin cutting was utilized. This room was connected with a large ice-box by

two openings whose size could be varied by movable slides. A small fan was placed in the upper opening and the fan was connected in series with a resistance box. Proper selection of size of the openings and the amount of resistance in series with the fan made it possible to maintain quite even temperatures in this room. As a matter of convenience the cats were fed their usual ration in the morning and the tests started 5 hours later. This procedure was followed in both series of animals. One month after the operation they were given hot box tests similar to those described by Teague and Ranson (1936).

The ability of the first group of animals to regulate their body temperature in a warm room improved with the lapse of time and was similar in all three. The record of cat 3 may be given as an example. An incubator temperature of 90° was necessary in order to maintain the rectal temperature of this cat within normal limits for the first 3 postoperative days. On the 4th PO (postoperative) day it was possible to lower the incubator temperature to 87°. On this same day this cat was taken out into the room at 79° for 3½ hours and the rectal temperature dropped to 97.1° in spite of constant shivering in those areas with intact nerve supply. On the 6th PO day exposure to a room temperature 79° for 6½ hours did not result in a fall in rectal temperature and on the 8th day 9 hours at 81° did not result in a drop. On the following day the animal was permanently removed from the incubator. Thereafter it was able to maintain normal body temperature in this room, the temperature of which averaged 82° and seldom changed more than 3° in any one day, the maximum variation being from 78° to 87° in the course of the experiment. As long as the temperature was about 85° there was occasional shivering in those areas whose nerve supply came from above the level of the cord section and as the room temperature dropped the shivering became more marked.

The temperatures at which panting began in hot box tests, similar to those described by Teague and Ranson (1936), were found in these 3 cats to be only slightly above the average for normal cats and well within the normal range of variation. Sweating was never observed.

The tests of ability to withstand cold are summarized in table 1. Since normal cats have no difficulty in maintaining the normal warmth of the body at temperatures of 35 to 40° (and probably at much lower temperatures) a failure to regulate at 65 to 70° represents marked impairment. The first test on cats 1 and 3 were made on the 16th and the last on the 65th PO days and the first test on cat 2 was made on the 26th and the last on the 75th PO day. Again the responses of cat 3 may be taken as typical for the group. In the second test, on the 22nd PO day, this cat's rectal temperature dropped from 101.1° to 98.8° after one hour in a room at 68°. By the 56th PO day there had been considerable recovery and the cat's temperature dropped only 2° (from 100.9° to 98.9°) as a result

of 5 hours' exposure to 68°. There was no further recovery in the next 9 days. It should be emphasized that this cat's weight continued to increase throughout this period when there was no further improvement in ability to regulate against cold.

Cats 1 and 2 did not show quite as marked recovery as did cat 3 for in the final tests it will be seen that the temperature of cat 2 dropped 5° to 97.4°, that of cat 1 dropped 4.5° to 96.8°, while that of cat 3 dropped only 2.6° to 98.4°. Usually the greater portion of the drop occurred in the first hour of the test so that changes in the last two hours were slight. Although skin temperatures were not measured the foot-pads and ears felt warm to the touch. The cats shivered violently in those regions with

TABLE 1

Cold box tests on cats with transections of the spinal cord in the lower cervical region

Rectal and room temperature in degrees Fahrenheit

DATE	CAT 1 RECTAL TEMPERATURE		CAT 2 RECTAL TEMPERATURE		CAT 3 RECTAL TEMPERATURE		COLD ROOM TEMPERATURE	ANIMAL ROOM TEMPERATURE	DURATION OF TEST
	Initial	Final	Initial	Final	Initial	Final			
5/23	102.5	98.8	101.9	97.4	100.8	96.6	65	83	1 hour
5/29	101.8	97.8	101.9	97.8	101.1	98.8	68	82	1 hour
6/5	101.7	97.4	101.3	96.9	100.1	97.9	68	83	2 hours
6/12	101.4	96.2	102.1	96.2	100.9	95.0	65	80	4 hours except cat 3, 2 hours
6/19	99.6	94.2	101.1	96.1	100.6	97.6	65	79	5 hours
6/25	101.9	96.7	101.2	98.0	101.0	99.0	70	81	5 hours
7/2	101.0	97.0	101.8	97.7	100.9	98.9	68	80	5 hours
7/9	100.9	95.5	101.9	97.0	100.9	98.6	68	83	5 hours
7/11	101.3	96.8	102.4	97.4	101.0	98.4	68	86	5 hours

intact nerve supply but shivering never occurred elsewhere. Erection of hair was never observed.

Second series. The cats of the second series were placed in a large incubator (set at about 90°). There they remained for 51 days when 3 were removed and placed in a large refrigerator which had been designed so that acclimatization of spinal cats to cold could be attempted. This box had been divided into two compartments by a vertical, insulated partition. The two compartments, each of which had its own door, were connected with openings at the top and bottom whose size could be varied at will. The cooling unit was in one compartment and 3 cats were placed on a support of stretched fish net in the other. The cool air as it entered the cat compartment was mixed with air from the top of the compartment and forced to the bottom by a small electric fan. Since the temperature

of the room in which the refrigerator stood was usually about 80 to 85° the compartment in which the cats were kept could be roughly regulated at any desired temperature from 77° to 50°. The first 3 animals were kept in the refrigerator for 1½ months. Then they were returned to the incubator and similar acclimatization was attempted in 2 other animals. After this the first 3 were returned to the refrigerator, etc. During the period that an animal was in the refrigerator it was found necessary to increase the diet 15 grams, giving 65 grams of ground beef hearts night and morning in order to prevent a loss in weight. At varying intervals tests were made of the ability of these animals to withstand cold. The majority of these were made by placing the animals in a room at 65° for 3 hours but in some cases lower temperatures and longer times were used. A few experiments were performed to determine if sweating occurred in response to heat and if shivering could be observed in regions whose innervation came from below the level of the cord transection.

In discussing the second series of animals cat 8 will be considered in detail. For the first 43 PO days it was kept in an incubator which for the first 3 days was set for 90° and then reduced to 85°. From day to day there was considerable variation in the incubator temperature which ranged from 87° to as low as 79°. During this period the cat's rectal temperature was usually within normal limits but on the one occasion when the incubator temperature dropped to 79° the morning temperature was 99.2°. On the 14th PO day the rectal temperature was 99.9° and the incubator temperature was 84°. After feeding the cat it was placed in the general animal room where the cage temperature was 74°. In 5 hours the rectal temperature had fallen to 94.6°. On the 44th PO day the animal was taken to the refrigerator, the temperature of which was slowly reduced from 77° to about 72°. The cat ran markedly subnormal temperatures, developed a diarrhea and was returned to the incubator on the 64th PO day. Nine days later it was again placed in the refrigerator which was now set at about 73°. For the first 3 days the cat would be left in the cold until its temperature had dropped to below normal levels and then it would be taken out into the room where the temperature was about 80-85°. When the cat's temperature had risen to normal it would be replaced in the refrigerator. After this period the animal remained in the refrigerator and its rectal temperature was usually about 99°. On the 81st PO day the cat's temperature dropped 3.2° to 96.3° as a result of 3 hours' exposure to 65°. The following day it was returned to the incubator where it remained for 32 days. The morning when it was placed in the incubator (average temperature 86°) its rectal temperature was 98.0° and that afternoon it had risen to 104.1° and it did not fall as low as 102° for 4 days (fig. 1). Thereafter the rectal temperature remained within normal limits. On the 102nd PO day 3 hours' exposure to 65° resulted

in a drop in rectal temperature of 3.6° to 96.8°. This final temperature was 0.5° higher than after the attempted acclimatization. On the 104th PO day the animal was returned to the refrigerator, the temperature of which was lowered from room temperature to 68 to 70° in 24 hours. The refrigerator temperature remained at this level for 18 days. During

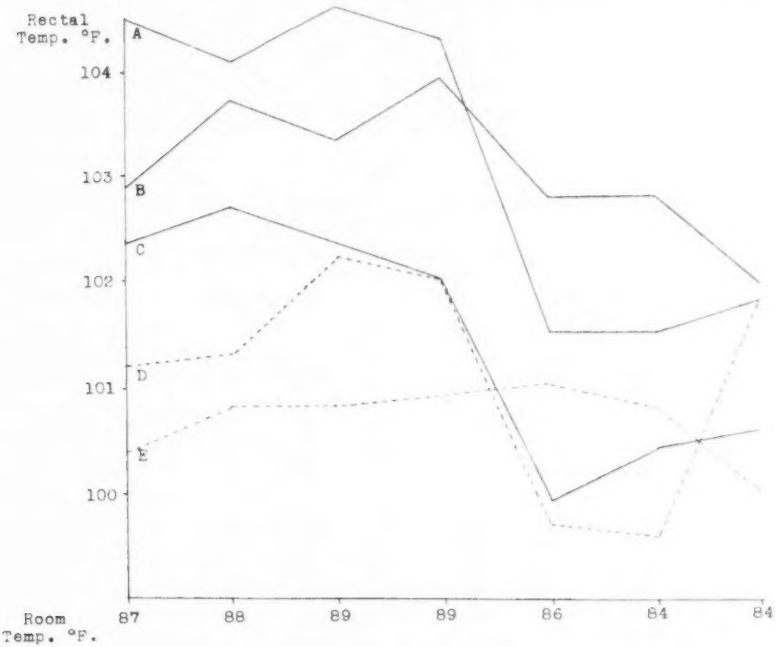

Fig. 1. Shows the effect of returning cats to the incubator after a period of acclimatization to cold. *A*, *B* and *C* represent the temperature records of 3 such cats (nos. 5, 7 and 8) based on daily observations made in the mornings. *D* represents the average morning temperatures of 3 cervical cats already acclimated to the incubator and *E* the average temperatures of 2 normal cats under the same conditions. Observe that for the first 5 days the temperatures of the cats acclimated to cold were consistently higher than that of the cats already accustomed to the heat. The wide changes in temperature of the cervical cats are in marked contrast to the almost constant temperature of the normal animals in the same room.

this period the cat's rectal temperature was usually about 96 to 97°. Then the refrigerator temperature was increased to 72 to 74° and the animal's temperature increased to 98 to 99°. This second attempt at acclimatization was successful for on the 137th PO day, after 33 days in the cold, 3 hours' exposure to 65° produced a drop of 2.4° to only 99.6°. After this the animal was kept in the general animal room (75–77°) where it

was able to maintain its rectal temperature within normal limits. At autopsy the section was found to be slightly caudad of the roots of C_7 and microscopic study of the scar revealed that there could be no question of the completeness of the transection.

In table 2 are summarized the results obtained from the second series of cats. Two tests are listed on each animal, i.e., the best test after acclimatization to cold and the best test after acclimatization to heat. By the best test is meant the test in which the final level of the rectal temperature was the highest. In all cases, except as noted in the table, the tests were for 3 hours at 65°. It will be seen that, while only one of

TABLE 2

Evidence of acclimatization in cats with transection of the spinal cord in the lower cervical region

Rectal temperature in degrees Fahrenheit

CAT NUMBER	BEST TEST AFTER ACCLIMATIZATION TO COLD		DAYS IN COLD	POST-OPERATIVE DAYS	BEST TEST AFTER ACCLIMATIZATION TO HEAT		DAYS IN HEAT	POST-OPERATIVE DAYS
	Initial temperature	Final temperature			Initial temperature	Final temperature		
4	102.3	100.0	21	110	101.8	95.9	87	87
5	101.9	99.3	35	147	102.6	98.8	10	100
6	103.0	100.6* 99.2†	11	168	101.9	99.4	10	122
7	101.2	100.1	46	155	102.0	96.6	8	94
8	102.0	99.6	33	135	100.4	96.8	21	100
9	100.6	99.6‡	18	149	99.3	97.5	135	135
10	100.0	97.9‡	18	149	99.9	97.2	112	112

* Three hours at 55°F.

† Six hours at 55°F.

‡ In animal room average temperature 75°F. These two animals were never kept in ice box.

the cats was able to maintain its rectal temperature above 99° after 3 hours' exposure to 65° when acclimatized to heat, 6 of the 7 animals were able to do so after acclimatization to cold. The exception, and one of the other cats, were never placed in the refrigerator but had been in the general animal room where the temperature averaged 75°. Cat 6 after acclimatization to cold was not seriously chilled by exposure to 55° for 6 hours.

As mentioned previously these tests of ability to prevent chilling were started 5 hours after feeding. An attempt was made to determine the influence of this short period between feeding and tests. Routine tests at 65° were made on cats 6 and 7 on the 186th and 183rd PO days respec-

tively. The rectal temperature of cat 6 remained within normal limits while that of cat 7 dropped 2.4° to 99.4°. On the following morning food was withheld and a cold test was started 16 hours after the previous evening's feeding. As a result of 3 hours' exposure to 65° the rectal temperature of cat 6 dropped 2.3° to 98.4° and that of cat 7, 5.4° to 97.2°. It is questionable which of the two tests truly represents the ability of these animals to withstand chilling. Bruhn (1940) found it necessary to withhold food for 24 hours in his metabolism studies of midbrain dogs.

Very shortly after these cats recovered from the anesthetic it was possible to elicit a crossed-extensor reflex. Throughout their survival period this reflex could consistently be obtained with only slight stimulation. In most of the animals it was not possible to elicit a scratch reflex until about 3 weeks after the operation, but after that it could be obtained easily. In those animals which survived 3 months or longer mass reflexes were often seen. A pinch of the tail or foot-pad, or a scratch on the side would produce at first a to-and-fro movement of the tail, a crossed extensor reflex or a scratch reflex respectively, then if the stimulus were repeated a few times, the activity would begin to spread, more and more muscle groups would become involved, and finally there would be vigorous twistings of the trunk, urination and occasionally defecation. After the spread had begun it was not necessary to continue the original stimulus. If the bladder or colon were distended it was much easier to secure this response. At first the nictitating membranes were relaxed but after a week or 10 days they returned to the usual position. At times, however, the nictitating membranes again relaxed and would remain relaxed for widely varying periods of time ranging from a few minutes to several days. This phenomenon could not be correlated with differences in the level of the lesion nor with the condition of the animal. The pupillary response to light was normal. Pinching the rear foot-pad produced little, if any, change in pupil size, but on the contrary, a light pinch of the fore pad caused a marked dilatation. This is in agreement with the work of Ury and Gellhorn (1939) who stress the importance of inhibition of the third nerve in reflex dilatation in response to pain. Maes (1939), who transected the spinal cord at C_1 and maintained the cats under artificial respiration on a heating pad for 3 to 6 hours, stated that he was able to elicit treading, raising of the pelvis and tail movements characteristic of "heat" as a result of tapping the perineum when the animals (previously treated with estrogens) were held in a crouching position. While elevation of the pelvis was not observed, the other responses could be obtained consistently upon tapping the perineum of this series of cats. This was done while the animals were lying on their sides and it is possible that if they had been held in a crouching position that elevation of the pelvis, also, would have occurred.

In order to secure greater accuracy in the observations on shivering than can be attained by the sense of touch (Sherrington, 1924) use was made of electrical recording. Electrodes embedded in Cambridge electrode jelly were applied to the skin overlying both extremities of the humerus and femur on one side. The cat's temperature was reduced to 95° by placing the animal in the cold compartment of the refrigerator at about 40°. Then the animal was placed in a shielded room and the electrodes connected with an amplifier and cathode ray oscillograph. In the muscles of the fore limb, whose innervation came from above the lesion the

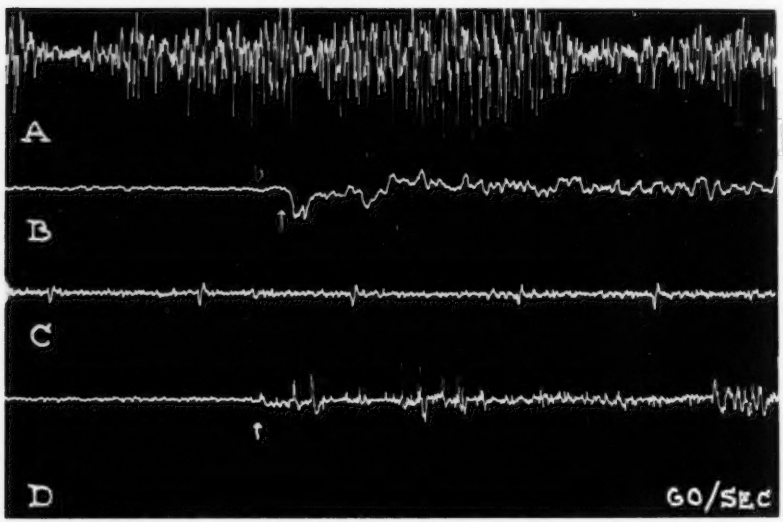

Fig. 2. Muscle potentials from the fore limb A and hind limb B of a cat with low cervical transection of the spinal cord with a rectal temperature of 95° and C from the fore limb and D from the hind limb of the same cat after its temperature had been raised to 101.6°. The arrows indicate pinching of the opposite hind foot and the muscle potentials which follow are associated with the crossed extensor reflex.

shivering was almost continuous (fig. 2A). The record from the hind limb (B) was taken a minute later. At the arrow the foot-pad was pinched and the ensuing activity is that of the crossed extensor reflex. Then a heating pad was placed beneath the cat and its temperature was raised to 101.6° and a record taken from the front leg (C) and from the hind leg (D). The periodic activity of the heart muscle is seen in C and the crossed extensor reflex is seen in D following the arrow which indicates pinching the foot. In the chilled animal the shivering which was so obvious in the record from the fore limb was absent in the hind limb

and it disappeared in the fore limb when the animal's temperature was raised.

Observations on sweating are rendered difficult because of evaporation which can completely mask a slow secretion. To obviate this difficulty fingers from rubber gloves were used to cover the cat's hind feet. Several normal cats were heated till panting occurred and in each case there was definite evidence of sweat inside the glove finger when it was removed. Four of the cervical cats were similarly tested and in no case was there any indication of sweat within the glove finger. Mass reflexes in patients with transected spinal cords are accompanied by sweating (Head and Riddoch, 1917) but in these cats, although mass reflexes accompanied by defecation and urination could be produced, sweating was never observed during these paroxysms.

Since cat 6 showed during the entire postoperative period considerably better reactions to cold than any of the others and was finally able to stand an exposure for 6 hours to a temperature of 55° and emerge from the test with a rectal temperature of 99.2° the question arises whether in this case the cord was completely cut. The cords from the second series of cats were fixed, embedded and cut in serial transverse sections and stained by the Van Gieson method. In none of the cords could evidence be found that the section had been incomplete and no bundle of uncut fibers could be traced across the scar. But in cats 4, 5, 6 and 7 the scar joining the severed parts of the cord together was so thin and irregular that it was equally impossible to be sure that no fibers crossed the scar joining the two ends together. If there were any such fibers they were few in number and could not be identified under the microscope. In the remaining 3 cats such a thick scar was found that there could be no question of the completeness of the cord section.

Since shivering never occurs in muscles whose innervation comes from below the level of the lesion and since erection of hair was never observed, there seem to be only two possible explanations for the acclimatization to cold. There must either be an increase in the basal metabolic rate or a chronic peripheral vasoconstriction. There is abundant evidence that changes in the thyroid gland occur as a result of exposure to cold (Uotila, 1939; Starr, 1940; Baillif, 1937) and that changes in the hypophysis may be seen (Baillif, 1938). This indicates that the rate of secretion of thyroxine may be increased as a result of long-continued exposure to cold. Indeed, Ring (1939) has recently shown that sufficient exposure to cold will increase the resting metabolism of white rats an average of 16 per cent. Since in the cold tests the ears and foot-pads of the cats acclimatized to cold did not seem perceptibly colder than those of cats from the incubator the other possibility—a chronic peripheral vasoconstriction—does not appear likely.

CONCLUSIONS

The results of these experiments show that cats with transection of the lower cervical cord are unable to make the adjustments necessary for maintaining a normal body temperature when there occurs a sudden and considerable fall in environmental temperature. But these animals are still capable of a limited slow adjustment to cold, an acclimatization. This increased ability to withstand cold which is acquired as a result of the gradual lowering of the environmental temperature is lost after the animals have been kept again in a warmer environment and is probably due to an increased metabolic rate.

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THE HEMOLYTIC ACTION OF CHYLE[1]

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It has been previously demonstrated that samples of lymph collected from the lacteals and thoracic ducts of dogs from 3 to 5 hours or more after the ingestion of fat are strongly hemolytic (Johnson and Freeman, 1938), by making ordinary red blood cell counts on mixtures of equal quantities *a*, of whole blood plus lymph[2] (about 0.015 cc. of each), and *b*, of blood plus Ringer's solution. Almost without exception the red blood cell counts made on blood and chyle mixtures were lower than the counts made on blood and Ringer's solution mixtures. The supernatant fluid of centrifuged samples of blood and chyle mixtures also showed definite evidence of hemolysis. These findings have been confirmed repeatedly, using the same technique with slight modifications.

What is this factor in chyle which causes hemolysis? The substance is found in the lacteals and thoracic duct only after fat-feeding. Lymph obtained from these sources in fasting animals or from cervical or foot lymphatics in fat-fed animals does not hemolyze red blood cells when mixed in equal quantities with whole blood. Thus, it is a substance which appears in the lymph only during the transport of products of fat absorption. The following substances were considered:

1. *Cholesterol.* This has been shown repeatedly to be antagonistic to the hemolytic action of such substances as fatty acids and saponin (Meyerstein, 1912; Brinkman, 1929). Therefore, it was not considered to be an important causative factor.

2. *Neutral fat.* This is probably not an important factor since mixtures of equal quantities of blood plus milk or cream give the same red blood cell counts as blood mixed with Ringer's solution.

3. *Bile salts.* Sodium taurocholate is definitely hemolytic and might be involved in the hemolytic action of chyle. Since good quantitative methods for bile salt determination are lacking, no chemical estimations were

[1] This work was in part aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2] Heparin was used as an anti-coagulant for both blood and lymph in some cases. In other instances, intravenous injection of Chlorazol Fast Pink BKS (Modell, 1939) prevented coagulation of both blood and lymph.

attempted. However, the introduction of bile or bile salts into the intestine yielded lymph possessing little or no hemolytic activity. Therefore, it seems that the rôle of bile salts in the hemolytic activity of chyle is probably insignificant.

4. *Osmotic pressure.* No direct measurements of the osmotic pressure of chyle were made in this study, but the work of several investigators (summarized by Drinker and Field, 1933) indicates that the total osmotic pressure of lymph is even slightly higher than that of serum. Furthermore, many mixtures of potently hemolytic chyle and whole blood showed crenation of the red blood cells. These findings suggest that salt osmotic pressure changes are not involved in the hemolytic action.

5. *Hydrogen ion concentration.* Determinations were made of hemolytic potency[3] and the pH of lymph samples collected under oil. The pH was determined by means of a glass electrode and was found to remain almost constant throughout an experiment, having no apparent relationship to hemolytic potency. Table 1 is a typical protocol.

TABLE 1

The hemolytic potency and pH of thoracic duct lymph collected after fat feeding

TIME AFTER FAT FEEDING	HEMOLYTIC POTENCY OF LYMPH	pH OF LYMPH
hours	*per cent*	
$3\frac{2}{3}$	20	7.357
4	34	7.357
$4\frac{1}{2}$	6	7.341
$4\frac{3}{4}$	3	7.349

6. *Temperature.* The temperature of the mixtures was kept constant without varying the results.

7. *Enzymes.* Chyle samples were collected and divided into two portions. One portion was heated to 75°C. for 10 minutes; the other was untreated. No constant or significant differences in hemolytic potency of heated and unheated samples were observed.

8. *Glycerol.* In an earlier report (Johnson and Freeman, 1938) it was suggested that glycerol might be responsible for the hemolytic action of chyle. Other investigators (Ramond and Flandrin, 1904) had demonstrated large amounts of glycerol in the lacteals and thoracic ducts of dogs,

[3] Throughout the paper, percent hemolysis is used as an expression of hemolytic potency of samples tested. It is determined by the formula $\frac{C_R - C_L}{C_R} \times 100$, where C_R is the control red blood cell count made upon mixtures of equal quantities of whole blood plus Ringer's solution and C_L is the red blood cell count made upon mixtures of equal quantities of whole blood plus the sample (usually lymph) whose hemolytic potency is being determined.

but they reported concentrations equally as high in the portal and peripheral blood streams, where presumably no hemolysis occurs. In the present study, glycerol was placed directly into washed, intact intestinal loops, or glycerol was fed with diets not containing fat in a number of dogs. This yielded thoracic duct lymph which was not hemolytic. These considerations seem to indicate that glycerol is probably not the substance which causes hemolysis.

9. *Free fatty acids and soaps.* Of the substances found in the gut during fat digestion, there remain free fatty acids and soaps. Might not quantities of these sufficient to cause hemolysis escape resynthesis into neutral fat and be absorbed into the lacteals? Several lines of evidence supported this hypothesis. Placing oleic acid or sodium oleate in washed intestinal loops of dogs gave intensely hemolytic chyle. Also, earlier workers (Faust and Tallqvist, 1907) fed fatty acid to one dog and a cholesterol ester to another, getting lymph with hemolytic power. They extracted the lymph with fat solvents, made a suspension of the dissolved material and showed that this also was hemolytic.

In an attempt to discover whether free fatty acid and soap were responsible for the hemolytic action of chyle, quantitative analyses for soap and free fatty acid were made. The analytic method employed combined features of methods published by several workers (Boyd, 1936; Bloor, 1915; Fowweather, 1926; Stoddard and Drury, 1929). Briefly, the method was as follows:

A 3:1 alcohol-ether mixture (both freshly redistilled) was acidified with 1 cc. of 3 per cent hydrochloric acid for each 50 cc. One cubic centimeter of the fluid containing fatty acid and soap was run slowly into 35 cc. of the mixture. This was heated to about 60°C. for two minutes, stirred to prevent superheating, cooled and centrifuged. The supernatant fluid was then poured into a 50 cc. volumetric flask and the residue was re-extracted with 15 cc. of the alcohol-ether. Two 20 cc. aliquots of the alcohol-ether extracts were placed in evaporating dishes containing extracted and washed sand and were evaporated to dryness with gentle heat on a steam bath. The dry residues were each dissolved in 25 cc. of petroleum ether and were filtered through fat-free filter paper. The duplicate samples of petroleum ether extract were heated almost to boiling and were titrated with approximately 0.02 normal sodium ethylate to a pink that did not change, using phenolphthalein as the indicator. Blanks, checks and knowns were run through the whole procedure.

Checks on the method consisted in adding known quantities of soap and fatty acid to lymph or Ringer's solution, and then analysing these solutions and emulsions as described above. The results are shown in figure 1, in which known milligrams added are plotted against milligrams detected by analysis. If the recovery were perfect, all the points would

fall on the straight line drawn into the figure. The distribution of the points shows that not quite all of the added soap or acid was recovered. However, the results were considered sufficiently accurate for the purposes of this study.

Next, the extent of hemolysis produced by mixing red blood cells with Ringer's solutions to which known amounts of fatty acid and soap were added in vitro was determined. These findings are recorded graphically in figure 2, in which the milligrams of added soap or fatty acid are plotted against the hemolytic potencies. Lines are drawn connecting determinations made on individual animals. Although the fluctuations from animal to animal are considerable, it is apparent that there is a positive correlation between quantity of fatty acid or soap added and the hemolytic potency of the solution or emulsion.

Finally, samples of thoracic duct lymph were analysed for soap and free fatty acid content. In fasting dogs, whose lymph showed no hemolytic activity, the quantities of soap and free fatty acid ranged from 1.0 to 2.0 mgm. per cubic centimeter—too little to produce detectable hemolysis. Values ranging from 3.3 to 6.3 mgm. per cubic centimeter were found in the course of $3\frac{1}{2}$ to $4\frac{1}{2}$ hours following fat-feeding. In a few instances, soap and free fatty acid were determined separately on each sample. The concentration of free fatty acid alone in chyle rarely reached 1 mgm. per cubic centimeter. Using somewhat different methods, other investigators have also found free fatty acids and soaps in chyle. Faust and Tallqvist (1907) found about 12 mgm. of soap plus free fatty acid in the thoracic duct lymph of a dog fed an oleic acid-cholesterol ester, and even more in a dog fed oleic acid. Munk (1880) also presents larger values for free fatty acid and soap than were found in the present study. Hoppe-Seyler (1879) calculated that from 2.5 to 4.0 mgm. of soap enter the blood stream by way of the chyle every minute following the ingestion of fat. The work of Freeman and Friedemann (1935) indicates that about 12 per cent of the fatty acids in chyle are uncombined. Lastly, Artom and Peretti (1935) found that 2 per cent of the fatty acids were present in chyle as soaps and free fatty acids.

Are the values reported here sufficient to account for the hemolysis observed? In each sample analysed chemically, the hemolytic potency was also determined. In figure 3 the results are plotted, showing the relationship of the quantity of soap or fatty acid detected in lymph to the hemolytic potency of the lymph. The line drawn in figure 3 is derived from the data shown in figure 2 by connecting the averages of the points on figure 2. Points in figure 3 lying above the line represent lymph samples containing at least enough soap or fatty acid to account for the extent of hemolytic potency observed. That the line in figure 3 is not placed too low is borne out by observations of Edwards (1939), McPhedran (1913), and Zinck, Clark and Evans (1922) who were able to produce definite

hemolysis with solutions of fatty acids and soaps even less concentrated than the solutions used in the present study.

The data in figure 3 seem to indicate that, in general, the quantity of soap and fatty acid found in chyle is adequate to account for the hemolytic potency. One can only speculate on the failure of the points to distribute themselves along a straight line. Perhaps the variations in the resistance of corpuscles from different animals to these agents are partly responsible. The data of figure 2 support this view. Perhaps there are variations in the anti-hemolytic action of serum, or in the concentrations of cholesterol, an anti-hemolytic agent. Several workers (Liebermann, 1907; Noguchi, 1907; Meyer, 1908; Meyerstein, 1912; Zinck, Clark, and Evans, 1922;

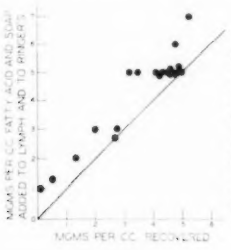

Fig. 1

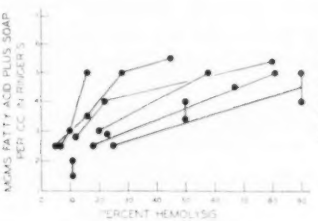

Fig. 2

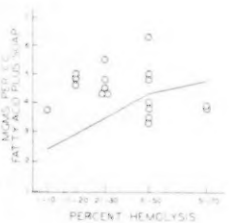

Fig. 3

Fig. 1. Relation of added milligrams of soap and free fatty acid to milligrams as detected by analysis. The straight line indicates the theoretical location of the points if the recoveries were complete.

Fig. 2. Relation of amount of soap or fatty acid added to Ringer's solution (in vitro) to extent of hemolysis observed when this was mixed with equal quantities of whole blood. Lines are drawn through determinations made on the cells of individual animals.

Fig. 3. Relation of hemolytic potency to quantity of fatty acid and soap determined by chemical analysis. The line drawn represents the averages of points plotted in figure 2.

Brinkman, 1929) have shown that serum exerts a protective action against hemolysis by soaps and fatty acids. The presence of some anti-hemolytic activity is suggested by the distribution of the points to the left, in figure 3, since these points represent chyle samples containing more than enough fatty acid or soap to account for the hemolytic potency. Finally, there may be variable quantities of insoluble calcium soaps formed (Brinkman and Szent-Gyorgyi, 1923).

Whether or not the figures reported are of significance, physiologically or pathologically, remains to be determined. It has been suggested (Johnson and Freeman, 1938) that absorption of these hemolytic agents into the lymphatics may be of adaptive value, since instead of entering capillaries at once, where red cells might be damaged, the hemolysins enter the blood stream only after they have been diluted by lymph from

many parts of the body. Further, the diluted hemolysins are poured into a stream of blood returning from many regions, instead of initially entering and mixing with the blood of the intestine alone. It remains to be demonstrated that the direct entrance of these substances into the capillaries of the portal circulation would destroy or damage red blood cells.

SUMMARY AND CONCLUSIONS

1. The existence of a hemolytic agent in thoracic duct lymph, during absorption of ingested fat, is amply confirmed. Other lymph is not hemolytic.

2. Evidence is presented that this hemolytic agent is not cholesterol, neutral fat, bile salts, enzymes, glycerol, or changes in osmotic pressure, hydrogen ion concentration, or temperature.

3. The soap plus free fatty acid content of chyle is 3.3 to 6.3 mgm. per cubic centimeter during rapid fat absorption. Most of this is probably soap.

4. These quantities are sufficient to account for the hemolytic action of chyle.

5. The duct lymph of fasting dogs contains too little fatty acid or soap to produce hemolysis.

6. The possible significance of these findings is discussed.

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THE ACTION OF IONS ON THE FROG HEART

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In studying the effect of varying the concentration of Na, K or Ca ion in Ringer's solution on the frog-heart rate, I have found that the heart rate is depressed if the concentration of any one of these ions is sufficiently greater or less than that generally considered normal for this solution. This depression is usually of a progressive nature and appears to be due simply to the abnormality, or toxicity, of these experimental Ringer's solutions. However, within certain limits of concentration, the heart continues to beat for long periods without any great change in rate (Spealman, 1938). These limits may tentatively be considered to be "physiologically normal."

My previous studies have concerned only the heart rate; and it is possible that the "normal" limits of concentration would be different for other activities of the heart. Unfortunately, most investigators who have made fairly extensive studies on the effect of ions on the cold-blooded heart have usually used concentrations which deviate widely from the values ordinarily used in Ringer's solution. (See Daly and Clark, 1921; Andrus and Carter, 1922, for the more recent, extensive studies.) It appears likely that they studied effects produced by rather abnormal solutions.

The present investigation was carried out to establish the approximate "normal" concentration limits of the different ions (Na, K, Ca and H) with respect to certain activities of the frog heart (the amplitude and duration of the ventricular response, the systolic and diastolic tone, and the a-v time interval) and to study the effect of varying the concentration of these ions, when kept within "normal" limits, on these activities.

METHODS. In all experiments, the heart was removed from the frog and perfused through the sinus venosus. The apex of the heart was attached to a heart lever. In the experiments on the amplitude of the ventricular response and on the tone (table 1), the extent of the excursions of the lever and the systolic and diastolic lengths of the heart were read from a millimeter scale held near the tip of the heart lever but not touching it; this procedure avoided the variable frictional load present when a lever is in contact with a surface. In the experiments on the a-v time interval and on the duration of the ventricular response (table 2), tracings

TABLE 1

The amplitude of the ventricular response and the tone in Ringer's solutions of various compositions

Table 1a gives a summary of the results obtained with Ringer's solution containing various concentrations of $CaCl_2$. The concentrations of $CaCl_2$ in mols per liter are given at the heads of the columns. The solutions otherwise have the composition of normal Ringer's solution. Tables 1b, 1c and 1d are constructed in the same manner and show the results obtained when the KCl concentration, the NaCl concentration, and the pH, respectively, were varied. Also included in table 1c are data obtained with Ringer's solution containing 0.1 mol per liter of dextrose (second column from right) and with a Ringer's solution containing half the normal concentration of NaCl and 0.1 mol per liter of dextrose (column at extreme right).

The amplitude of the ventricular response is expressed as a decimal fraction of the value obtained in normal Ringer's solution. For each experimental value of the amplitude of the ventricular response, two other figures are given; the upper-right figure represents the change in systolic tone, and the lower-right figure, the change in diastolic tone. ((+) indicates an increase and (−) a decrease in tone.) The numerical values given for the tone changes were obtained by dividing the change in length of the heart by the amplitude of the ventricular response. (See text for a more complete explanation.) In the experiments marked with an asterisk, the heart rate was maintained constant at 40 beats per minute by electrical stimulation; in the other experiments the heart was allowed to beat at its own natural rate. 0.00 indicates the heart stopped contracting before measurements could be made. — indicates that no value was taken.

a. $CaCl_2$

EXP.	0.00025	0.0005	0.001	0.002 (N. RINGER'S)	0.003	0.004	0.008
1	—	−0.33 0.65 +0.02	−0.13 0.87 0.00	0.00 1.00 0.00	+0.09 1.06 +0.03	+0.09 1.12 −0.03	—
2	—	−0.44 0.56 0.00	−0.37 0.63 0.00	0.00 1.00 0.00	—	+0.16 1.16 0.00	—
3	—	−0.19 0.81 0.00	—	0.00 1.00 0.00	—	+0.04 1.04 0.00	—
4	—	−0.25 0.77 −0.02	—	0.00 1.00 0.00	+0.15 1.13 +0.02	—	—
5	—	−0.33 0.67 0.00	—	0.00 1.00 0.00	+0.03 1.03 0.00	+0.12 1.12 0.00	—
6*	−0.57 0.30 +0.13	−0.24 0.76 0.00	—	0.00 1.00 0.00	—	−0.04 0.95 +0.01	−0.04 0.89 +0.07

TABLE 1—*Continued*

a. $CaCl_2$—*Continued*

EXP.	0.00025	0.0005	0.001	0.002 (N. RINGER'S)	0.003	0.004	0.008
7*	−0.21 0.58 +0.21	−0.14 0.81 +0.05	—	0.00 1.00 0.00	—	−0.07 0.99 −0.06	−0.10 0.79 +0.11
8*	−0.48 0.45 +0.07	—	−0.08 0.92 0.00	0.00 1.00 0.00	—	−0.03 0.95 +0.02	−0.07 0.87 +0.06

b. KCl

EXP.	0.00025	0.0005	0.001	0.002 (N. RINGER'S)	0.004	0.008	0.016
1	—	—	−0.25 0.77 −0.02	0.00 1.00 0.00	−0.12 0.86 +0.02	—	—
2	—	—	−0.05 0.95 0.00	0.00 1.00 0.00	0.00 0.99 +0.01	—	—
3	—	—	+0.02 1.02 0.00	0.00 1.00 0.00	0.00 1.03 −0.03	—	—
4	—	—	−0.18 0.88 −0.06	0.00 1.00 0.00	0.00 1.04 −0.04	—	—
5*	—	−0.06 0.85 +0.09	−0.07 0.92 +0.01	0.00 1.00 0.00	+0.06 1.00 +0.06	−0.23 0.73 +0.04	—
6*	−0.15 0.85 0.00	+0.10 1.08 +0.02	+0.06 1.06 0.00	0.00 1.00 0.00	−0.05 0.98 −0.03	−0.02 1.00 −0.02	−0.06 0.82 +0.12
7*	−0.20 0.59 +0.21	−0.01 0.84 +0.15	+0.03 1.00 +0.03	0.00 1.00 0.00	—	+0.01 1.00 +0.01	−0.45 0.38 +0.17

c. NaCl

EXP.	0.05	0.10 (NORMAL RINGER'S)	0.13	0.20	0.10 + 0.10 M DEXTROSE	0.05 + 0.10 M DEXTROSE
1	—	0.00 1.00 0.00	+0.07 1.06 +0.01	—	—	—

TABLE 1—*Concluded*

c. NaCl—*Continued*

EXP.	0.05	0.10 (NORMAL RINGER'S)	0.13	0.20	0.10 + 0.10 M DEXTROSE	0.05 + 0.10 M DEXTROSE
2	—	0.00 1.00 0.00	+0.21 1.13 +0.08	—	+0.15 1.10 +0.05	—
3	—	0.00 1.00 0.00	+0.11 1.11 0.00	—	+0.05 1.10 −0.05	—
4	—	0.00 1.00 0.00	+0.14 1.14 0.00	—	−0.12 0.90 −0.02	—
5*	−0.34 0.52 +0.14	0.00 1.00 0.00	−0.04 0.99 −0.03	−0.11 0.69 +0.20	−0.02 0.98 +0.00	−0.06 0.94 0.00
6*	−0.21 0.68 +0.11	0.00 1.00 0.00	+0.04 1.02 +0.02	−0.14 0.75 +0.11	−0.03 0.96 +0.01	−0.13 0.84 +0.03
7*	−0.18 0.49 +0.33	0.00 1.00 0.00	+0.01 1.03 −0.02	−0.15 0.69 +0.16	+0.02 1.01 +0.01	+0.06 1.04 +0.02

d. pH

EXP.	3.0	4.3	5.8	7.0	7.7 (N. RINGER'S)	8.3	9.6	10.6
1*	0.00	—	—	−0.01 0.95 +0.04	0.00 1.00 0.00	—	—	—
2*	—	—	—	—	0.00 1.00 0.00	−0.04 0.98 −0.02	—	0.00
3*	—	−0.62 0.26 +0.12	—	−0.03 1.00 −0.03	0.00 1.00 0.00	+0.03 1.02 +0.01	—	−0.18 0.64 +0.18
4*	—	—	−0.13 0.85 +0.02	+0.02 1.02 0.00	0.00 1.00 0.00	—	−0.13 0.88 −0.01	−0.17 0.65 +0.18
5*	—	—	−0.12 0.86 +0.02	−0.01 0.98 +0.01	0.00 1.00 0.00	—	−0.13 0.87 0.00	—

TABLE 2

The a-v time interval (A-V) and the duration of the ventricular response (DVR) in Ringer's solutions of various compositions

The column at the extreme left shows the concentration of the substances studied in mols per liter; otherwise each solution has the composition of normal Ringer's solution. All values obtained in these experiments are expressed as decimal fractions of the values obtained in normal Ringer's solution. Average values are given in the column at the extreme right. The dash (—) indicates that no value was taken with that solution in the experiment.

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3	EXPERIMENT 4	EXPERIMENT 5	AVERAGE
a. $CaCl_2$						
0.0005						
A-V	0.94	0.93	0.82	—	1.06	0.94
DVR	0.85	0.81	0.84	—	0.72	0.81
0.001						
A-V	—	1.00	0.83	1.11	0.98	0.98
DVR	—	0.84	0.86	0.91	0.85	0.87
0.002*						
A-V	1.00	1.00	1.00	1.00	1.00	1.00
DVR	1.00	1.00	1.00	1.00	1.00	1.00
b. KCl						
0.001						
A-V	1.61	1.21	1.77	1.20	1.37	1.43
DVR	1.33	1.03	1.10	1.20	1.22	1.18
0.002*						
A-V	1.00	1.00	1.00	1.00	1.00	1.00
DVR	1.00	1.00	1.00	1.00	1.00	1.00
0.004						
A-V	0.90	1.03	0.78	0.69	0.71	0.82
DVR	0.86	0.74	0.76	0.94	0.64	0.79
c. NaCl						
0.100*						
A-V	1.00	1.00	1.00	1.00	1.00	1.00
DVR	1.00	1.00	1.00	1.00	1.00	1.00
0.125						
A-V	1.02	0.91	0.95	0.94	1.00	0.96
DVR	1.00	0.97	1.25	1.00	1.00	1.04
0.100†						
A-V	1.25	1.02	0.91	—	1.07	1.06
DVR	1.21	1.37	1.38	—	1.28	1.31

TABLE 2—*Concluded*

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3	EXPERIMENT 4	EXPERIMENT 5	AVERAGE
			d. pH			
6.9						
A-V	1.03	1.01	1.03	—	1.00	1.02
DVR	1.00	0.98	0.91	—	0.97	0.97
7.7*						
A-V	1.00	1.00	1.00	1.00	1.00	1.00
DVR	1.00	1.00	1.00	1.00	1.00	1.00
8.4						
A-V	0.94	0.94	—	1.00	1.00	0.97
DVR	1.00	1.12	—	0.90	1.03	1.01

* Normal Ringer's.
† + 0.10 M/L dextrose.

were made on a constant speed kymograph. In these experiments a lever was also attached to one of the atria. The a-v time interval was determined by measuring the distance on the drum between the upstroke of the two levers; the duration of the ventricular response was determined by measuring the distance between the point at which the lever started to rise and a more or less arbitrary point at which the lever returned to the base line.

The procedure in all experiments was to make the necessary measurements or tracings, first, with the heart perfused with normal solution, then with the heart perfused with the experimental solution, and finally with the heart again perfused with normal solution. In the experiments on the amplitude of the ventricular response and on the tone, 3 measurements were made at 5 minute intervals with each solution; in the experiments on the a-v time interval and on the duration of the ventricular response, 3 measurements were made at 10 minute intervals with each solution.

In most of the experiments, the heart was allowed to beat at its normal rhythm. In some of the experiments (marked with asterisks in the tables) the heart was driven by means of electrical stimulation at a rate of 40 beats per minute; in these experiments, the sinus venosus was removed from action by tying the cannulating ligature on the atrial side of the white crescentic line. The reason for using artificial stimulation in some of these experiments was to rule out the possibility that a change in the heart rate was responsible for some of the effects found; for it is known that the heart rate affects certain other activities of the heart, e.g., the amplitude of the ventricular response.

Each value reported in the tables for the amplitude of the ventricular

response, for the duration of the ventricular response, and for the a-v time interval was calculated by dividing the average of the values obtained with the experimental solution by the average of the values obtained with normal Ringer's before and after perfusion with the experimental solution.

The values given for the tone changes require some comment. The readings actually taken were the positions of the tip of the heart lever on the millimeter scale during complete systole and complete diastole. An elevation of the position of the lever, in either systole or diastole, occurring during perfusion with an experimental solution is termed plus (+) in the table; a depression is termed minus (−). These correspond respectively to an increase in tone (decrease in length of the heart) and to a decrease in tone (an increase in length of the heart). In order to show quantitatively the changes in systolic and diastolic lengths, the actual differences in millimeters between the systolic positions and between the diastolic positions of the lever during perfusion with normal Ringer's solution and during perfusion with the experimental solution are divided by the amplitude of the ventricular response (extent of excursion of the lever) occurring during perfusion with normal Ringer's solution. The figures so obtained show the quantitative changes in the systolic and diastolic lengths of the heart which result in a change in the amplitude of the ventricular response. For example, in table 1a, the figures under the column headed 0.0005 M/L Ca ion in experiment 1 signify that the amplitude of the ventricular response occurring with that solution was 0.65 the amplitude of the ventricular response occurring with normal Ringer's solution, i.e., there was a decrease in the amplitude of contraction of 0.35 of the original amplitude. Of this decrease, 0.33 was due to an increase in systolic length of the heart and 0.02 was due to a decrease in diastolic length of the heart.

RESULTS. Table 1 shows the effect on the amplitude of the ventricular response and on the systolic and diastolic tone (length) of varying the Na, K, and Ca ion concentrations, the pH, and the osmotic pressure. The table shows that the amplitude of the ventricular response becomes smaller if the concentration of any of these ions deviates too far from normal. With concentrations closer to normal, the heart is not greatly affected except by Ca ion which causes an increase in the amplitude of the ventricular response and an increase in systolic tone as the concentration of this ion is increased.

Table 2 shows the effect on the a-v time interval and on the duration of the ventricular response produced by variation of the Na, K, and Ca ion concentrations, the pH, and the osmotic pressure. The various ions were studied only in concentrations close to normal. This was done because the heart will not maintain a constant beat outside this range, and it is undesirable to use artificial stimulation. The table shows that K ion causes a decrease in the a-v time interval and a decrease in the duration of the ventricular response as the concentration of this ion is increased.

Ca ion causes an increase in the duration of the ventricular response as the concentration of this ion is increased. Addition of dextrose (increasing the osmotic pressure) caused an increase in the duration of the ventricular response. The heart is not significantly affected in these respects by the other ions.

Discussion. When the concentration of any one of the positive ions in Ringer's solution is sufficiently different from that usually used in normal Ringer's solution, the amplitude of the ventricular response decreases. This decrease appears to be associated with an increase in systolic length and a decrease in diastolic length of the heart; the result is that there is less difference between the contracted and the relaxed lengths of the heart. Because these changes are not related to any particular ion, it is probable that this behavior is simply a non-specific response of the heart to abnormal or toxic solutions. A further fact supporting this conclusion is that this behavior of the heart is usually progressive; that is, when the heart is perfused with one of the solutions which cause this behavior, the systolic and the diastolic lengths of the heart become more nearly the same as time goes on. Finally, as I have previously mentioned, in studying the effects of ions on the heart rate, I have found that outside of certain limits of concentration, the rate usually became progressively less (Spealman, 1938). These limits are practically the same as the concentration limits (which may be inferred from table 1) outside of which the contractile ability of the heart shows evidences of failing. I think it is justifiable to conclude that the concentration limits suggested in table 1 of this publication (also see Spealman[1], 1938) can be considered as the approximate "normal" limits of concentration for each of these ions for the perfused frog-hearts used here (Rana pipiens, winter frogs).

There have been but few studies carried out within these "normal" limits. However, the effect of Ca ion on the amplitude of the ventricular response has been studied over the "normal" range by Clark (1928), and it is well known that increasing the concentration of this ion increases the amplitude of the ventricular response as table 1 shows. In fact McLean and Hastings (1934) have used this as a method for determining ionized calcium in mammalian blood. This increase is due entirely to a decrease in the systolic length of the heart.

It is usually stated that changes in K ion concentrations also affect (in the opposite direction to Ca) the amplitude of the ventricular response. In fact, Trendelenburg (1921) gives curves showing this. It is likely that the decrease in amplitude of the ventricular response found by Trendelenburg was obtained only by K ion concentration sufficiently high to be

[1] In this publication, the values for Na ion concentration are too high by 0.016 M/L. This was due to the fact that the $NaHCO_3$ concentration was taken to be 0.0175 M/L; the concentration actually was 0.00175 M/L.

"abnormal." As mentioned above, a decrease in the amplitude of the ventricular response can be obtained easily by sufficiently increasing or decreasing the concentration of any of the ions of Ringer's solution. I found no change in the amplitude of the ventricular response when K ion concentration was varied in the region of its normal value.

Ca ion seems to be the only factor studied which, in reasonable concentration limits, affects the amplitude of the ventricular response or the tone. The studies with Na ion deserve some comment, for an additional factor, the osmotic pressure of the solution, becomes important here. I have varied the Na ion concentration 1, by changing the NaCl concentration (which also allows the osmotic pressure to vary), and 2, by decreasing the NaCl concentration and adding dextrose in sufficient concentration to maintain the osmotic pressure (see column to extreme right of table 1c). In neither case was the amplitude of the ventricular response or the tone greatly changed within "normal" limits. (The first 4 experiments with 0.13 M/L NaCl show some increase in the amplitude of the ventricular response; however, this effect was not obtained in the 3 experiments in which artificial stimulation was used. The moderate effect in the first 4 experiments is probably not significant.) Addition of dextrose to normal Ringer's solution (to increase the osmotic pressure) did not greatly affect these properties of the heart. It might be expected that the amplitude of the ventricular response in the experiments without artificial stimulation would be changed somewhat, since dextrose decreases the heart rate; for it is well known that decreasing the heart rate may affect the amplitude of contraction. In 2 of these 3 experiments there is an increase in the amplitude of the ventricular response; but in the 3 experiments where artificial stimulation was used, there was no effect on the amplitude of contraction. It may be concluded that neither Na ion concentration nor osmotic pressure appreciably affect these properties of the heart.

As table 2 shows, the duration of the ventricular response becomes greater as the K ion concentration is decreased, and, to a smaller extent, as the Ca ion concentration is increased. The Ca ion action may be simply secondary to the effect of Ca ion on the amplitude of the ventricular response; for it is reasonable to expect that if the muscle contracts more completely, it will take a longer time for the response to occur.

Increasing the osmotic pressure with dextrose also increases the duration of the ventricular response to some extent. This is probably due to the fact that the heart rate is decreased by dextrose; for I have found (unpublished results) that the duration of the ventricular response is increased as the heart rate is decreased (artificial stimulation).

K ion appears to be the only factor studied here which appreciably affects the a-v time interval. As my experiments show, the a-v time interval is decreased as the K ion concentration is increased.

CONCLUSIONS

1. All positive ions ordinarily included in Ringer's solution must be present within certain concentration limits in order that the perfused heart can function properly for a long period of time. Outside these limits all ions produce rather similar depressive changes in the heart activities studied. These changes appear to be non-specific, and due to the fact that the solutions are too abnormal to allow the heart to function properly.

2. Certain activities of the heart are specifically modified when the concentrations of certain positive ions are varied within what may be termed their "normal" limits. An increase in Ca ion concentration causes an increase in the amplitude of the ventricular response, an increase in systolic tone, and an increase in the duration of the ventricular response. An increase in K ion concentration causes a decrease in the a-v time interval and a decrease in the duration of the ventricular response. The activities of the heart investigated here are not appreciably affected by variation within "normal" limits of the Na ion concentration, the osmotic pressure, or the pH.

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THE EFFECT OF CLIMATE UPON THE VOLUMES OF BLOOD AND OF TISSUE FLUID IN MAN

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The blood and tissue fluid constitute important links in the chain of mechanisms by which man regulates his body temperature. It is reasonable to expect, therefore, that changes of climate may cause marked variations in the volume of either the blood or tissue fluid and also changes in their composition. Barcroft (1, 2, 3) while on his way to the Peruvian Andes found that the blood volume (calculated from cell volume measured with CO) increased about 35 per cent in three members of this party while they were sailing through the tropics, and decreased again as they got further south to cooler weather. More recently, Bazett (4) has reported increases of 30 to 40 per cent in the blood volume (calculated from the plasma volume measured with vital red) as the summer heat struck Philadelphia, and 8 to 48 per cent in six subjects who, in the wintertime, spent a few days in a room at 32°. In the course of some studies during the past summer in the hot and humid "Delta" region of Mississippi, in the town of Benoit, halfway between Memphis and Vicksburg, we were able to make 65 determinations of plasma volumes and of "available fluid" in three groups of individuals. The first group consisted of ten laboratory workers who were measured both in Boston and Mississippi, the second of white sharecroppers native to Mississippi, and the third of colored sharecroppers native to Mississippi. In addition, we made a few measurements on colored boys and on less active adults.

The techniques used were essentially those of Gregersen and Stewart (5) and of Gibson and Evelyn (6). However, as we made some slight modifications, our method will be described briefly. The subject came to the laboratory in the morning fasting and lay down for half an hour. Four cubic centimeters of blood were then drawn from the antecubital vein, and 1 gram of NaSCN dissolved in 20 cc. of water was injected intravenously, followed immediately by 15 mgm. of the dye T1824 dissolved in 10 cc. of water. Four blood samples of 4 cc. each were then drawn at approximately 15-minute intervals, usually from the opposite antecubital vein, followed by two more samples at hourly intervals, the

subject remaining on the bed. The blood was transferred to a hematocrit tube of 4 cc. capacity and containing 1 drop of heparin solution. (This contained 0.8 per cent NaCl and 400 units of heparin (Connaught Laboratories, Toronto) per cc.) The samples were centrifuged for an hour, and the concentration of dye in the plasma determined on the Evelyn photoelectric colorimeter. For the determination of the NaSCN, 1 cc. of the plasma was then squirted rapidly from an accurately calibrated syringe into 10 cc. of 10 per cent trichloroacetic acid and the precipitated proteins were removed by filtering. Eight cubic centimeters of the filtrate were added to 3 cc. of 10 per cent acid ferric nitrate and the resulting color read within a minute or two. It was observed at the high temperatures and under the rather intense light prevailing in the laboratory in Mississippi that the color faded sufficiently rapidly to produce errors if the solutions were allowed to stand over 5 minutes after the color had developed.

The calculations for the plasma volume and the available fluid followed the lines laid down by Gregersen (5) and Gibson (6), but the blood volume was calculated from the plasma volume and the hematocrit without making the rather large correction suggested by Gregersen to allow for the greater proportion of plasma in the capillary blood. The "available fluid" was calculated directly from the concentration of NaSCN in the plasma from the last three samples of blood, i.e., those taken at one hour, two hours, and three hours after the injection. The "interstitial fluid" was calculated by subtracting from the "available fluid" the plasma volume + 70 per cent of the cell volume (the cells being about 70 per cent water). These terms and figures are arbitrary and take no account of the uncertain but probably small amount of NaSCN which enters certain cells, e.g., those of the salivary glands. They are the same terms and figures that Gregersen and Stewart used, with the one important exception noted above, namely, that we made no correction for the increased proportion of plasma in the capillary blood. Our reasons for omitting this correction are that its proper value is not known, that we feel Gregersen's figure is too large, and that as many other authors, especially Barcroft (2) and Gibson (6), have not made this correction our figures will be more readily comparable with theirs if we omit it. The work of Manery and Hastings (7) suggests that it may not be possible to divide tissues into those which do take up NaSCN and those which do not, but rather into those which take more and those which take less. It is probable therefore that our values run high rather than low.

Judging from the consistency of the values obtained from different samples in the same experiment and also from repeated determinations on the same individual, the probable error of the method was about 2 per cent for the plasma volume and between 1.5 per cent and 2 per cent for the available fluid. In many experiments the probable error was less

than this, but there were also many in which the plasma, though clear to the eye, gave readings on the colorimeter suggestive of a slight cloudiness, perhaps attributable to a large meal rich in fat the preceding evening. In these the probable error was 2 per cent to 4 per cent for the plasma volume, but the available fluid determinations were not affected. On the other hand, though repeated determinations of available fluid on one subject under the same conditions gave fairly consistent results, there was a disquieting amount of difference between individuals and big changes with changed conditions. It is possible that these variations may be due in part to changes in the permeability of certain cells to NaSCN as well as to changes in the amount of interstitial fluid.

The climate in this region of Mississippi during the summer is uniform, hot, and damp. In the period between June 6 and August 16, during which our observations were made, the highest temperature was 101°F. and the lowest 68°F. The mean of the daily highs was 93°F. and the mean of the lows 73°F. These were the outdoor temperatures; indoors the fluctuation was undoubtedly less. The mean humidity at 6:30 a.m. was 88 per cent, at noon 59 per cent, and at 6:30 p.m. 65 per cent. The average precipitation was 2.8 inches per month and it came principally as thunder-showers. The "percentage of sunshine" was 65. These figures for temperature and rainfall are taken from the United States Weather Bureau data for Greenville, Mississippi, a town about twenty miles south of Benoit and very similar in climate. The humidity and sunshine figures are averages of the observations made at Vicksburg and Memphis, the nearest cities on each side of Benoit at which such observations are made, and similarly situated with respect to the river.

Our results with respect to blood and plasma volumes agree qualitatively but not quantitatively with Barcroft's and with Bazett's. They are given in the tables but may be summarized as follows. Of the ten Whites who had determinations made upon them in Boston (or elsewhere in the North) during the winter (November through April inclusive), nine lost weight, on the average 2 kgm., and one gained 1 kgm. on going to Benoit. In some individuals part of this loss occurred before reaching Mississippi. In five the hematocrit reading increased, in one it was unchanged, in four it went down, the average increased insignificantly. The plasma volume increased in eight and decreased in two subjects, the greatest increase being 420 cc. and the greatest decrease 245 cc. The average went up 4.2 per cent in contrast with Barcroft's 35 per cent and Bazett's 30 per cent. The average plasma volume per kilogram and per square meter of body surface increased a little more (because of the loss of weight), 6.1 per cent and 4.7 per cent respectively. Since the average hematocrit value was practically unchanged, the percentage change in blood volume was almost the same as in plasma volume. In general, those individuals who showed the largest

increases in plasma volume showed decreases in their hematocrit readings, while those whose plasma volume increased but little or decreased in the hot weather showed increases in their hematocrit readings.

There was no consistent change in plasma volume between the first set of determinations, which were made within ten days of arrival, and the second set made a month later. Some individuals went up, some down.

TABLE 1

Plasma volumes in Boston, Massachusetts, and in Benoit, Mississippi

	AGE	HEIGHT	BODY WEIGHT, KILOGRAMS			PLASMA VOLUME, CC.			PLASMA VOLUME, CC. PER KGM. OF BODY WEIGHT			PLASMA VOLUME, LITERS PER M². OF BODY SURFACE		
			Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ
A. Ten White laboratory workers														
Highest	48	188	84.0	79.0	+1.0	3,845	3,880	+420	55.3	52.9	+6.9	2.11	1.98	+0.21
Lowest	26	165	61.5	61.2	−5.0	2,970	3,230	−245	40.1	45.6	−3.2	1.67	1.81	−0.13
Average	34.3	175	74.6	72.6	−2.0	3,436	3,581	+145	46.4	49.3	+2.8	1.82	1.90	+0.086
			% change		−2.7	% change		+4.2	% change		+6.1	% change		+4.7
B. Seven White sharecroppers in Mississippi														
Highest	21	186		71.5			3,900			58.5			2.01	
Lowest	17	170		53.5			3,110			46.9			1.75	
Average	18.9	177.3		64.6			3,410			53.0			1.89	
C. Twenty-one Colored sharecroppers in Mississippi														
Highest	24	181		72.3			3,900			59.2			2.14	
Lowest	17	165		55.3			2,870			44.4			1.59	
Average	20.1	174		61.9			3,300			53.6			1.89	
D. Two Colored servants in Mississippi														
Highest	23	194		77.9			4,450			57.2			2.13	
Lowest	22	178		74.1			3,950			53.3			2.06	
Average	22.5	186		76.0			4,200			55.3			2.10	
E. Four Colored boys from sharecroppers' families in Mississippi														
Highest	11	154		33.7			2,010			60.0			1.68	
Lowest	8	142		32.9			1,900			57.8			1.58	
Average	9.5	148.3		33.4			1,953			58.8			1.63	

The average plasma vol./kgm. rose slightly due to the loss of weight. The figures in the tables are average values.

In comparing individuals with one another, the volumes per square meter of surface area proved to be more constant than the volumes per height or weight. This agrees with Rowntree and Brown (8). Though there were considerable variations in the plasma vol./m². within a group, the average variation of individuals from the mean of their group being

4.6 per cent, there was a remarkable constancy in the means for the groups. Thus the laboratory Whites, who increased 4.7 per cent, or from 1.82 liters plasma vol./m^2. in Boston to 1.90 liters in Mississippi, were in Mississippi within 1 per cent of the values for the native Negroes and the native Whites, which were both 1.89 liters. This extremely close correspondence is no doubt fortuitous, but it indicates the improbability

TABLE 2

Blood volumes in Boston, Massachusetts, and in Benoit, Mississippi

	SURFACE AREA		HEMATOCRIT			BLOOD VOLUME, CC.			BLOOD VOLUME, CC. PER KGM. OF BODY WEIGHT			BLOOD VOLUME, LITERS PER M^2. OF BODY SURFACE		
	Boston	Mississippi	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ
A. Ten White laboratory workers														
Highest	1.99	1.97	47.6	47.2	+2.8	6,540	7,055	+605	91.9	97.3	+13.1	3.50	3.56	+0.40
Lowest	1.70	1.70	39.8	41.4	−4.0	5,560	5,950	−200	75.2	81.4	−2.4	2.90	3.20	−0.10
Average	1.896	1.876	44.0	44.05	+0.05	6,125	6,400	+275	82.8	87.9	+5.1	3.24	3.40	+0.16
			% change		+0.1	% change		+4.5	% change		+6.2	% change		+4.9
B. Seven White sharecroppers in Mississippi														
Highest		1.94		47.9			7,290			111.0			3.75	
Lowest		1.61		41.6			5,560			85.0			3.19	
Average		1.801		45.2			6,237			97.1			3.46	
C. Twenty-one Colored sharecroppers in Mississippi														
Highest		1.89		46.5			6,740			111.0			3.89	
Lowest		1.60		39.7			5,130			80.0			2.89	
Average		1.752		43.0			5,782			93.9			3.31	
D. Two Colored servants in Mississippi														
Highest		2.09		44.4			8,000			103.0			3.83	
Lowest		1.92		41.9			6,800			91.8			3.56	
Average		2.00		43.2			7,400			97.4			3.70	
E. Four Colored boys from sharecroppers' families in Mississippi														
Highest		1.23		38.4			3,260			97.4			2.65	
Lowest		1.16		35.8			3,034			90.8			2.54	
Average		1.20		37.4			3,115			93.7			2.60	

that under the conditions of our experiments there were any changes in blood volume comparable to those found by Barcroft or Bazett. Our average values are higher than Gibson's (9) general averages, but agree with his figures for athletic individuals.

The discrepancy between the small change we observed and the large ones previously reported is hard to explain. Barcroft measured the cell volume by the CO method. Bazett in most cases measured the plasma

volume by the vital red method, which is essentially the same as ours except for the kind of dye used. He also used CO in a few experiments and T1824 in others. Both calculated the blood volume from the hematocrit readings. The former of these methods gives lower results than the latter (fewer cells in the capillary blood? very rapid removal of a small part of the dye?), but the change with climate should be the same with either method, and so it is in Barcroft's and Bazett's work. It is possible that there is a sudden increase followed by an equally sudden decrease. Bazett (4) has observed some indication of this, but we found no consistent difference between our values obtained within a few days of arrival and those a month later. It is true, however, that, though some of our measurements were made within three days of arrival in Mississippi, the subjects had been exposed to a few days of hot weather on the way down and conceivably might have had an increase in blood volume which had passed off. It is more likely that individual variation plus a different sort of activity may have caused the difference. In our experiments the subjects were for the most part doing medium to hard physical work and losing weight. From the accounts of the other experiments, the subjects may have been taking life easily and gaining weight. There is another possibility, namely, that the difference in results depends upon the dye used. Unlikely as this seems it is suggested by the fact that in the two experiments in which Bazett et al. used T-1824 the increases were relatively small (8 per cent and 11 per cent), and lie within the range of our observations.

The changes in available fluid were a little larger but no more consistent. Seven individuals went down and three up, but the rises were less than the falls and the average decreased 1.17 liters, or 6.9 per cent, from the Boston level of 16.87 liters. The interstitial fluid shows an even greater drop, 1.38 liters, or 12.0 per cent, from the Boston average of 11.55 liters, but again eight decreased while two increased. It might be pointed out that one of the pair whose interstitial fluid moved in the opposite direction from the other eight was the same individual whose plasma volume decreased instead of increasing, and that the other member of the pair was a frequent visitor to Mississippi and accustomed to its climate. There is a close correlation between the weight lost by an individual and the loss of interstitial fluid. If the subjects are arranged from 1 to 10 in order of their weight loss, the order of their loss of interstitial fluid per square meter of body surface runs as follows: 1, 2, 3, 4, 6, 7, 5, 10, 9, 8. Eight is the consistently atypical individual. The "rank-order" correlation is +0.89. The loss of weight exceeded the loss of interstitial fluid by 0.6 kgm. on the average, but the individual variation was great. There was a moderate degree of positive correlation between the available fluid per square meter in the different groups and the amount of sweating during a standard

piece of work, but no correlation between the efficiency and the available fluid. The interstitial fluid appeared to be less closely correlated with the sweating than the "available fluid," which includes the blood, but the number of subjects was not sufficient to be sure of this.

There was a striking difference between the white and colored groups in the amount of interstitial fluid per square meter of body surface. The

TABLE 3

Available fluid and interstitial fluid in Boston, Massachusetts, and in Benoit, Mississippi

	AVAILABLE FLUID, LITERS			AVAILABLE FLUID, LITERS PER M². OF BODY SURFACE			INTERSTITIAL FLUID, LITERS			INTERSTITIAL FLUID, LITERS PER M². OF BODY SURFACE			RATIO OF INTERSTITIAL FLUID TO BLOOD VOLUME		
	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ	Boston	Mississippi	Δ
A. Ten White laboratory workers															
Highest	20.80	17.50	+2.15	10.65	9.31	+1.19	15.11	12.20	+2.36	7.75	6.49	+1.30	2.31	1.93	+0.43
Lowest	12.50	13.35	−4.05	7.35	7.18	−2.12	7.70	8.05	−4.45	4.53	4.32	−2.24	1.38	1.31	−0.94
Average	16.87	15.70	−1.17	8.89	8.34	−0.55	11.55	10.17	−1.38	6.08	5.40	−0.68	1.89	1.59	−0.30
	% change		−6.9	% change		−6.2	% change		−12 0	% change		−11.2	% change		−15.6
B. Seven White sharecroppers in Mississippi															
Highest		19.30			9.95			13.03			6.72			1.79	
Lowest		12.10			6.85			6.56			3.69			1.00	
Average		14.81			8.19			9.43			5.20			1.51	
C. Twenty-one Colored sharecroppers in Mississippi															
Highest		22.80			13.20			17.97			10.37			3.24	
Lowest		15.30			8.23			9.10			5.00			1.68	
Average		16.84			9.61			11.74			6.71			2.06	
D. One Colored servant in Mississippi															
		18.80			9.80			12.86			6.69			1.89	
E. Four Colored boys from sharecroppers' families in Mississippi															
Highest		9.20			7.92			6.48			5.58			2.06	
Lowest		8.80			7.15			5.36			4.47			1.81	
Average		9.00			7.52			6.01			5.03			2.01	

Whites, both the laboratory workers while in Mississippi and the native white workmen, were about the same, but only one of them had as high a value as the average for the Negroes, and only one Negro was lower than the average for the Whites. Since the Negroes were more efficient than either group of Whites in doing the standard work, we thought that there might be a correlation between the efficiency and the amount of fluid, but, as mentioned above, there was none.

Since the blood volume went up upon going to Mississippi and the interstitial fluid went down, it was evident that the greatest change would be found in the ratio of the interstitial fluid to the blood, i.e., the ratio of the fluid outside the blood vessels to that inside them. This is given in the right-hand columns in table 3. The average decrease in this ratio was nearly 16 per cent, though again two individuals went up while eight went down.

There is a pronounced difference between the Whites and the Negroes, though the northern Whites while in the North are not far below the value for the Negroes. The colored boys showed the same ratio as the colored adults, though on account of their relatively larger surface area their interstitial fluid values per square meter were out of line with the values on the colored adults and even below the white adults.

SUMMARY

A group of ten white laboratory workers on moving to a hot, damp climate for the summer showed on the average a small increase in the volumes of both blood and plasma, both absolute and relative to body weight and to surface area. The average change was +5 per cent but the range was from −6 per cent to +12 per cent.

The interstitial fluid, defined as the fluid outside the cells and outside the blood vessels, decreased in the heat 11 per cent on the average but the range was from −34 per cent to +26 per cent.

There was no difference between the colored sharecroppers, the white sharecroppers, and the laboratory workers while in Mississippi in respect to plasma volume per unit of surface area but the interstitial fluid volume was 25 per cent higher in the Negroes than it was in the white sharecroppers or the white laboratory workers.

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RELATION OF MUSCLE ELECTROLYTE TO ALTERATIONS IN SERUM POTASSIUM AND TO THE TOXIC EFFECTS OF INJECTED POTASSIUM CHLORIDE[1]

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Previous work has shown that potassium enters and leaves the cells of voluntary muscle under varying circumstances. In this laboratory it has been shown that muscle potassium is increased in the terminal stages of adrenal insufficiency and following total nephrectomy (1). Heppel has found that feeding a diet low in potassium to young growing rats resulted in marked losses of muscle potassium (2). We have independently confirmed Heppel's observations in our study of adult rats. Prolonged tetanic stimulation of skeletal muscle has been shown by Fenn (3) to produce a loss of potassium from the muscle and Baetjer (4) has found that depriving muscle of oxygen will also decrease muscle potassium.

The present work was undertaken to determine what electrolyte changes occur in serum and muscle when potassium enters or leaves muscle cells of intact normal animals. We were also interested in determining whether the capacity of skeletal muscle to take up potassium altered the toxic effects of this cation.

EXPERIMENTAL PROCEDURES. Adult male rats were used in all experiments and were fed on Purina Dog Chow (called stock diet) except when diets with altered content of sodium and potassium were sought. The special diets had the following proportions of basic ingredients: commercial lactalbumin, 18 grams; sucrose, 25 grams; commercial dextrin, 32 grams; vegetable fat (Crisco), 22 grams; cod liver oil, 1 gram; yeast powder, 2 grams; and bone ash, 2 grams. By adding sodium chloride or potassium chloride, the "high K," "low K," and "low Na" diets were made. The various diets were analyzed for Na and K and their composition is given in table 1.

The rats continued to hold or to gain weight on each diet except that some of the heavier ones on the "low K" diet lost some weight when first put on the diet. The initial loss was either regained or further loss stopped after a few days. A few of the rats which were left on the "low K" diet

[1] Aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

for two to three months finally became sick and lost weight. None of these animals died spontaneously although some were maintained as long as 115 days before being killed. Food and water were offered to all rats up to the time of sacrifice, except that when potassium chloride was injected, food and water were withdrawn from the cages during the period of injection.

As will be brought out in the presentation of data, a number of rats received intraperitoneal injections of potassium chloride in a solution containing 200 mM. of KCl per liter. Except in group IX, which received a single fairly large injection, the solution was given in several small injections at intervals of one to two hours starting at 9 a.m. and continuing until 5 p.m. or until the animals were killed. When injections were carried out for more than one day, the rats received none overnight, an interval of about 17 hours.

In group IV a solution containing NaCl 1.2 per cent and sodium bicarbonate 0.4 per cent was injected intraperitoneally. This solution

TABLE 1

Composition of diets by analysis

	mM PER 100 GRAMS	
	K	Na
Stock	15.5	23.7
High K	25	1.7
Low K	1.6	17.7
Low Na	16.6	1.7

was likewise given at intervals of one to two hours during a period of fasting.

All animals were anesthetized with ether before being killed by withdrawing as much blood as possible from the abdominal aorta. Tissue was removed and analyzed as described in a previous paper (5). In the tables serum Na and Cl are expressed per liter of ultrafiltrate of serum, using the dried weight for water and a Gibbs-Donnan factor of 0.96. Serum potassium is expressed per liter of serum. Tissue analyses are expressed per 100 grams of fat-free solids except potassium. In the case of this cation the tissue concentrations have been reduced to intracellular potassium by subtracting extracellular potassium. The latter value was assumed to be measured by the concentration in serum and the extracellular volume of water. Extracellular water was measured by the ratio of tissue chloride to the concentration of chloride in the ultrafiltrate of serum.

EXPERIMENTAL RESULTS. *I. Variations in muscle potassium occurring with normal serum electrolyte.* Table 2 shows that the muscle potassium

of adult rats may vary from 44 to 50 mM. per 100 grams of fat-free solids at times when the concentration of potassium in the serum is normal. Rats in groups I and V had muscle potassium values in the upper half of this range, i.e., from 47 to 50 mM. per 100 grams of fat-free solids and rats in groups II, III and IV had values in the lower half, i.e., from 44 to 47 mM. Rats in group I were fed the stock diet of Purina Dog Chow

TABLE 2

Muscle potassium accompanying normal serum potassium concentrations

GROUP	DIET	NUMBER OF RATS	SERUM			MUSCLE					
			Per liter serum ultrafiltrate		Per liter serum	Per 100 grams fat-free solids					
			Na	Cl	K	Na	Cl	K*	P	Protein	H_2O
			mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*grams*	*cc.*
I	Stock	13	146.9 ±0.74	113.0 ±0.87	4.0 ±0.21	9.99 ±0.18	7.2 ±0.15	48.8 ±0.18	33.4 ±0.35	92.3 ±0.56	340.5 ±1.90
II	"High K"	9	147.6 ±0.83	118.3 ±1.37	4.2 ±0.19	10.6 ±0.33	7.0 ±0.23	45.2 ±0.36	31.9 ±0.37	95.4 ±0.56	337.4 ±2.66
III	Stock	10	148.1 ±0.93	112.9 ±0.88	4.6 ±0.33	9.3 ±0.25	6.7 ±0.25	46.9 ±0.33	32.6 ±0.28	97.2 ±0.64	329.6 ±1.71
IV	Stock	4	141.2 ±2.29	114.5 ±1.26	4.8 ±0.26	10.9 ±0.36	7.6 ±0.20	45.9 ±0.72	32.6 ±0.34	97.0 ±0.82	341.8 ±2.86
V	"Low Na"	5	149.4 ±1.72	112.2 ±0.08	4.8 ±0.04	10.3 ±0.002	7.6 ±0.02	48.3 ±0.51	32.8 ±0.06	95.1 ±0.54	343.0 ±2.21

Group I. Control group.

Group II. On diet 24 to 51 days. Half of group were also given NaCl in water with the same result.

Group III. Injected with 3 to 4.6 meq. KCl per 100 grams of rat in 72 hours. Survived 18 hours after last injection.

Group IV. Injected with 4.8 to 12.1 meq. Na per 100 grams of rat in 1.2% NaCl and 0.4% $NaHCO_3$ solution over a period of 72 to 96 hours. Survived 18 hours after last injection.

Group V. On diet 16 days.

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

and the muscle potassium of these rats may be considered as representing the usual values for normal healthy adult rats. Rats in group V were fed a diet similar in its potassium content to the stock diet but low in sodium. Since the muscle potassium of rats in group V was similar to that of rats in group I, the decrease in muscle potassium of rats in group II, some of which were also fed a diet low in sodium, is not dependent on their low sodium intake. The fact that half of the rats in group II were

given added sodium chloride in their drinking water without producing significant differences in their muscle potassium from the other half of the group is further indication that the decrease of the muscle potassium of rats in group II to the low normal range was in some way associated with the high potassium intake in the diet. Further support for the concept that muscle potassium may actually be lowered by a high potassium

TABLE 3

Muscle potassium accompanying increased serum potassium concentrations

GROUP	DIET	NUMBER OF RATS	SERUM			MUSCLE					
			Per liter serum ultrafiltrate		Per liter serum	Per 100 grams fat-free solids					
			Na	Cl	K	Na	Cl	K*	P	Protein	H_2O
			mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*grams*	*cc.*
I	Stock	13	146.9 ±0.74	113.0 ±0.87	4.0 ±0.21	9.99 ±0.18	7.2 ±0.15	48.8 ±0.18	33.4 ±0.35	92.3 ±0.56	340.5 ±1.90
VI	Stock	7	143.4 ±0.54	121.4 ±0.75	7.5 ±0.20	8.2 ±0.26	7.3 ±0.14	51.7 ±0.52	34.5 ±0.29	95.9 ±1.03	353.0 ±1.54
VII	Stock	5	145.2 ±0.86	120.0 ±1.14	5.5 ±0.23	8.7 ±0.30	7.2 ±0.19	49.5 ±0.36	33.2 ±0.18	94.8 ±1.28	346.3 ±2.82
VIII	Stock	4	143.2 ±0.81	128.0 ±1.41	14.7 ±0.37	8.3 ±0.48	9.7 ±0.43	53.1 ±0.96	32.0 ±0.59	92.9 ±1.43	359.2 ±1.08
IX	Stock	8	137.0 ±3.21	115.3 ±2.47	12.5 ±2.07	7.5 ±0.29	7.4 ±0.30	49.2 ±0.24	31.9 ±0.21	95.1 ±0.30	356.6 ±7.08

Group I. Control group.

Group VI. Injected 1.1 to 1.4 meq. KCl per 100 grams of rat 6 to 7 hours and sacrificed 15 to 30 minutes after last injection.

Group VII. Injected 1.1 to 1.3 meq. KCl per 100 grams of rat 6 to 7 hours and sacrificed 60 to 90 minutes after last injection.

Group VIII. Injected 1.5 to 3.0 meq. KCl per 100 grams of rat 8 to 29 hours and sacrificed 15 to 40 minutes after last injection.

Group IX. Injected 0.5 to 2.0 meq. KCl per 100 grams of rat in one injection and sacrificed within 30 to 60 minutes.

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

intake is found in the results observed in rats in group III. These animals were repeatedly injected intraperitoneally with small amounts of potassium chloride over a period of three or four days and sacrificed eighteen hours after the last injection. From data that will be presented in table 3 it is certain that the injected rats had high serum and muscle potassiums during the period of injections, but that during the interval between the

last injection and the time of sacrifice the muscle potassium not only returned to its original levels (47 to 50 mM. per 100 grams of fat-free solids) but actually decreased further to low normal values (44 to 47 mM.). It is important to note that at least at the time of sacrifice the concentration of potassium in the serum of rats in group III was within normal limits. We emphasize this point because it will subsequently be shown (table 4) that decreases in muscle potassium below 44 mM. per 100 grams of fat-free solids were invariably associated with significant decreases in the concentrations of serum potassium. The decrease in muscle potassium to low

TABLE 4

Serum and muscle electrolyte of rats with muscle potassium below normal. Results of injecting KCl into rats with abnormally low muscle potassium

GROUP	DIET	NUMBER OF RATS	SERUM			MUSCLE					
			Per liter serum ultrafiltrate		Per liter serum	Per 100 grams of fat-free solids					
			Na	Cl	K	Na	Cl	K*	P	Protein	H_2O
			mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*grams*	*cc.*
I	Stock (Purina Dog Chow)	13	146.9 ±0.74	113 ±0.87	4.0 ±0.21	9.99 ±0.18	7.2 ±0.15	48.8 ±0.18	33.4 ±0.35	92.3 ±0.56	340.5 ±1.90
X	"Low K" (Control)	6	147.5 ±1.07	106 ±1.52	2.5 ±0.24	15.1 ±1.50	6.6 ±1.80	37.6 ±1.88	31.6 ±0.49	96.6 ±1.97	332.8 ±2.69
XI	"Low K" (Injected KCl)	6	145.2 ±1.07	119.8 ±3.00	8.3 ±1.79	11.3 ±0.67	7.5 ±0.44	46.9 ±0.35	32.6 ±0.35	95.5 ±0.62	338.3 ±2.20

"Low K" X averaged 57 days on diet.

"Low K" XI averaged 47 days on diet. Injected 2.6 to 3.7 meq. KCl per 100 grams of rat over a period of 26 to 48 hours. Survived 1 to 17 hours after the last injection.

* Corrected for extracellular K.

All concentrations represent mean result plus or minus the standard error.

normal levels (44 to 47 mM.) can be produced by other methods than increasing the intake of potassium. Similar decreases in muscle potassium were observed in rats in group IV that were repeatedly injected intraperitoneally with relatively large amounts of a mixture of NaCl and Na HCO_3 over a period of three to four days.

The fact that the concentrations of serum potassium in table 2 do not show significant differences does not preclude the possibility that as the muscle potassium varies between 44 and 50 mM. per 100 grams of fat-free solids there may be changes in the concentration of serum potassium. Subsequent tables show that fluctuations in muscle sodium and potassium

may be quite rapid following significant alterations in the concentration of serum potassium. The data in table 2 merely indicate that at the time of sacrifice the concentrations of potassium in the serum were normal. In addition to finding normal concentrations of potassium in the serum in table 2 we observed that the concentrations of sodium and chloride in the serum likewise were within normal limits except for a somewhat high chloride in the serum of rats in group II and a low sodium in rats in group IV. It was also observed that the muscle sodium and chloride of rats in all groups in table 2 did not differ significantly, giving further support to the concept that the range of muscle potassium between 44 and 50 mM. per 100 grams of fat-free solids may be looked upon as a range of normal physiological adjustment of muscle electrolyte.

Balance experiments were conducted in a few rats in groups III and IV and indicated that the low muscle potassium of these rats could be explained by potassium diuresis.

II. Variations in muscle potassium accompanying increased concentration of serum potassium. The analyses in table 3 show that while potassium probably readily enters muscle cells, it is difficult to produce sustained high values. In group VI the rats were given small doses of KCl intraperitoneally each hour for 6 to 7 hours and then killed 15 to 30 minutes after the last injection. All of these animals showed high muscle potassium and slightly low muscle sodium while serum potassium was moderately high. However, if the animals were treated in the same way but killed after 60 to 90 minutes, as in group VII, muscle potassium returned to the normal level and serum potassium was only slightly elevated. None of the animals in groups VI and VII showed symptoms of potassium poisoning.

If larger doses of potassium are given, symptoms of potassium poisoning develop. These symptoms appear within 10 to 20 minutes and if death does not occur in about 50 minutes after an injection recovery gradually ensues. Group VIII represents animals obviously about to die spontaneously as the result of repeated large doses of potassium chloride. While the muscle potassium is higher than in group VI, the striking difference is in the high serum potassium. However, single large doses of potassium chloride in group IX did not raise muscle potassium to abnormal levels although serum values are at levels known to produce electrocardiographic changes and almost as high as in group VIII.

Attention is directed to the low values for muscle sodium in all rats injected with potassium chloride.

III. Variations in muscle potassium produced by diets low in potassium. In contrast to the difficulty in sustaining muscle potassium above the normal level is the readiness with which muscle rendered deficient in potassium retains potassium injected intraperitoneally. In table 4,

group X shows the muscle values in rats fed the diet low in potassium. In each of the rats of this group both the serum and muscle potassiums are abnormally low, while the muscle sodium is obviously high. Since muscle chloride is not high, the increase in muscle sodium must be intracellular rather than extracellular. The changes in muscle sodium and potassium become more marked the longer the rats are kept on the diet deficient in potassium. The lowest value for muscle potassium (29 mM.) was obtained on a rat kept on the diet for 115 days.

Before injection with potassium chloride group XI was treated like group X and undoubtedly had muscle potassiums of the same order of magnitude. The higher muscle potassium in group XI than in group X was brought about by the injection of potassium chloride and the potassium which went into the muscle cells remained in the muscle at least 17 hours. This is contrary to the experience in groups VI, VII and III, when potassium rendered abnormally high returns to high normal levels within 60 to 90 minutes after the last injection and to low normal levels, i.e., 45 mM. per 100 grams of fat-free solids, if 18 hours elapses after the last injection.

By balance experiments it was found that injecting potassium chloride leads to a retention of potassium in rats previously fed a diet low in potassium while a negative balance was found by a similar injection in rats fed a normal diet.

It will be noted that muscle sodium returned toward normal when muscle potassium was raised in group XI.

IV. Interchangability of muscle sodium and potassium. As was shown by Heppel (2) and pointed out by us in the previous paragraphs, there is evidence of a reciprocal relationship between muscle sodium and potassium. Since from quantitative considerations extracellular potassium cannot be involved in this interrelationship, the values involved must be intracellular sodium and potassium. Since sodium is both intracellular and extracellular, a first approximation of the value for intracellular sodium was calculated as follows:

$$\frac{(Cl)_t}{[Cl]_s} = (H_2O)_e$$

$$(Na)_t - [Na]_s \times (H_2O)_e = (Na)_i$$

in which $(Cl)_t$ represents total tissue chloride; $[Cl]_s$ is the concentration of chloride in an ultrafiltrate of serum; $(Na)_t$ is sodium of tissue: $[Na]_s$ is the concentration of sodium in an ultrafiltrate of serum; $(Na)_i$ is intracellular sodium and $(H_2O)_e$ is extracellular water. It must be borne in mind that, since some muscle chloride is not diffusible (6), the extracellular water calculated by the ratio $\frac{(Cl)_t}{[Cl]_s}$ is somewhat too large. Hence intra-

cellular sodium, $(Na)_i$, is too small. The error is probably fairly constant although its exact magnitude is not known. This error accounts for the appearance of a negative value for intracellular sodium in some instances.

Figure 1 shows the relationship between this calculated intracellular sodium and muscle potassium. It will be seen that there is a good inverse relationship. Apparently for each 2 mM. of potassium that leaves the cells, about 1 mM. of sodium enters the cells. The negative values indicate that the calculated value for intracellular sodium is about 2 mM.

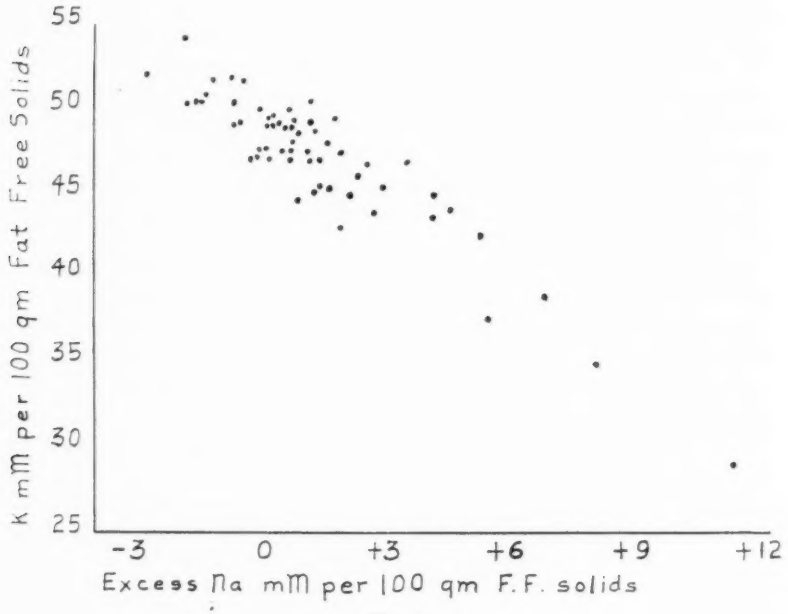

Fig. 1

too small. This assumption fits in with the evidence that about 1 mM. of chloride is associated with connective tissue (7) and is not diffusible (6).

V. The relation of muscle potassium to the toxic effect of injected potassium. In all groups of rats injected with KCl toxic effects were obtained in certain instances. With large doses of KCl the rats began to breathe rapidly within ten to fifteen minutes; this dyspnea rapidly increased and the animals became disinclined to move when disturbed; terminally they became cyanotic and pale. Death occurred in 5 to 40 minutes after the onset of symptoms. Apparently if the dose of KCl did not kill within 40 minutes, recovery gradually occurred. At death the typical dilated heart

of potassium poisoning was observed. From observation it was possible to predict the outcome and grade the degree of toxic effect. Table 5 was arranged to demonstrate the relation between the toxic effect of the injected KCl and the potassium level in the muscle and serum.

In general the severity of the toxic symptoms seems to be directly associated with the level of serum potassium. When the serum potassium was less than 10 mM. per liter, no symptoms or only a moderate degree of toxicity were noted at the time of sacrifice. In all instances in which

TABLE 5

Relation of toxic effect of injected KCl to muscle potassium

DIET	NUMBER OF EXPERIMENT	K ABSORBED* FROM PERITONEUM	PERIOD OF SURVIVAL	CLINICAL CONDITIONS	SERUM POTASSIUM	MUSCLE POTASSIUM
		meq./100 grams of rat	*minutes*		*meq./liter of serum*	*meq./100 grams fat-free solids*
"Low K"	6	0.75	40 (killed)	Not sick	5.9	43.7
"Low K"	9	0.77	55 (killed)	Not sick	7.9	42.0
Stock	7	0.39	45 (killed)	Moderately sick	8.7	49.0
Stock	8	0.50	55 (killed)	Moderately sick	8.7	50.1
Stock	14	0.52	40 (killed)	Very sick; dyspneic, breathing fast, apathetic	13.6	49.6
Stock	11	0.66	40 (killed)	Very sick; dyspneic, breathing fast, apathetic	14.2	49.6
Stock	12	0.89	35 (died)	Convulsions	19.9	49.8
Stock	13	1.28	17 (died)	Convulsions	21.8	49.1
"Low K"	10	1.17	48 (died)	Convulsions	21.3	44.3
"Low K"	15	1.39	55 (died)	Convulsions	20.5	37.1

* K absorbed equals the amount of K remaining in peritoneal fluid as actually determined at times of sacrifice subtracted from the measured amount of K injected intraperitoneally.

serum potassium was as high as 20 mM. per liter, spontaneous death occurred or was about to occur. As shown by experiments 6 and 9, rats on a "low K" diet were strikingly less susceptible to the toxic effect of KCl than rats on the stock diet (expts. 7 and 8) despite the fact that a larger dose of KCl was absorbed from the peritoneal cavity. It is noteworthy that, despite the absorption of more KCl, the muscle and serum potassium are lower in experiments 6 and 9 than in experiments 7 and 8. In the case of experiments 14 and 11, the increased toxicity on smaller doses of KCl is marked. Although the rats on "low K" diet are more

resistant, it is possible to produce fatal effects as is shown in experiments 10 and 15. The difference in reaction in this case is brought out by the longer period of survival. It will be noticed that low muscle potassium did not protect the rats from the effects of the rapidly rising serum K which had reached 20 mM. at the time of death.

Discussion. The data have been presented chiefly from the point of view of the factors which bring about variations in muscle potassium and the effects of these variations on susceptibility to the toxic manifestations following injection of potassium chloride. The other tissue analyses are included to demonstrate that the variations in muscle potassium are not necessarily accompanied by changes in the other factors excepting intracellular sodium. There are significant variations in protein per 100 grams of fat-free solids. Presumably increase in protein indicates decrease in some other fat-free solids rather than increase in protein. Changes in glycogen could account for the alterations in protein per 100 grams of fat-free solids shown in the tables. Since the changes in potassium are not accompanied by constant changes in protein, variations in muscle potassium are not accounted for by variations in non-protein fat-free solids (glycogen?). Since phosphorus is fairly constant, variations in muscle potassium and intracellular sodium do not apparently alter the cellular content of phosphorus compounds.

The variations in tissue water are in part accounted for by variations in the concentration of extracellular sodium and in part by variations in sodium and potassium of the muscle. Nevertheless the relationships between various factors of the tissue which might express osmotic pressure and the concentration of sodium plus potassium in the ultrafiltrate of plasma are quite variable. This result is explained in part by the fact that many of the analyses represent conditions in serum and muscle at a time when rapid adjustments are being made. For instance, evidences of shift of both water and sodium out of the cell are obtained within fifteen minutes after the injection of potassium chloride into the peritoneal cavity. Also within sixty to ninety minutes after muscle potassium has been elevated to abnormally high values, the concentration in muscle is restored to normal (see groups VI and VII). Hence while muscle water undoubtedly tends to increase when sodium and potassium are retained in muscle (6), the present data do not give a satisfactory expression of these relationships.

Additional observations support the idea that muscle potassium ranging from 44 to 47 mM. per 100 grams of fat-free solids must be regarded as normal although the usual values vary from 47 to 50 mM. For example, occasionally rats on a normal diet and otherwise not subjected to any unusual strain show values for muscle potassium at the lower level. The same observation has been made on cats (6). Furthermore, it is rather

easy to produce in a short time the lower concentration of muscle potassium. When values below 44 mM. actually have been produced, abnormally low concentrations of potassium in serum have almost invariably been encountered. Similarly concentrations of muscle potassium above 50 mM. always are accompanied by high concentrations of potassium in serum. Nevertheless there is no obligate relationship between the concentration of potassium in serum and the concentration of potassium in muscle.

The inverse relationship between intracellular sodium and potassium that has been shown to exist in the present study applies equally well to animals in adrenal insufficiency and in nephrectomized rats (1). The exchange of potassium for sodium in the muscle cell in these animals does not cover so wide a range as we found in the present study since the variation in muscle potassium was not so extreme. The replacement of potassium by sodium in the muscle cells has also been demonstrated in this laboratory to occur in rats injected with desoxycorticosterone (8). So far we have not been able to ascertain any conditions where this inverse relationship does not exist providing the exchange is large enough to be detected by the methods used.

Previous work has shown that evidences of potassium poisoning can be closely correlated with the level of serum potassium (9). In turn the concentration of serum potassium has been shown to affect cardiac rhythm, contraction and tone (9). The present experiments are entirely in accord with previous work emphasizing the importance of the concentration of serum potassium in determining the onset of potassium intoxication. However, the present data demonstrate, in addition, that low levels of muscle potassium delay the onset of toxic elevation of serum potassium and permit larger doses of potassium to be given without evidences of potassium poisoning. The data show why adrenalectomized animals that manifest defective renal excretion of potassium and tend to have high muscle potassium are peculiarly susceptible to the toxic effect of this cation.

SUMMARY

The usual range of muscle potassium in adult rats is 47 to 50 mM. per 100 grams of fat-free solids. Muscle potassium levels as low as 44 mM. may occur even though the concentration of potassium and sodium in the serum and the amount of sodium in the muscle remain normal.

Increases in muscle potassium above 50 mM. produced by injecting KCl are transitory in normal intact animals and return to high normal levels (49 mM.) within sixty to ninety minutes after the last injection and to low normal levels (45 mM.) eighteen hours after the last injection. Such transitory increases in muscle potassium are accompanied invariably by a shift of sodium out of the cell and have not been demonstrated except

in the presence of an increased concentration of serum potassium. With the fall in muscle potassium which follows the transitory rise, the serum potassium concentration and muscle sodium return towards normal levels. Significant increases in muscle potassium occurred without evidence of potassium poisoning in animals in which the serum concentration of potassium was not elevated above 10 meq. per liter.

Increases in muscle potassium in animals whose muscle has previously been rendered deficient in potassium (i.e., from 29 to 44 mM. per 100 grams of fat-free solids) remain fixed at least for seventeen hours after the last injection of KCl. The tolerance of animals with low muscle potassium to the toxic effects of injected potassium is appreciably greater than the tolerance of animals with normal muscle potassium. It is suggested that the slower rise in serum potassium concentrations in the former is due to the greater capacity of the skeletal muscle to take up potassium from extracellular fluid. The toxic effects of injected potassium are directly related to the elevation in concentration of serum potassium and only indirectly to that of muscle potassium.

Within the range of muscle potassium between 29 and 55 mM. per 100 grams of fat-free solids found in these experiments there is a reciprocal relationship between muscle potassium and the intracellular sodium of the muscle. It was found that about 1 mM. of sodium may be interchanged for 2 mM. of potassium.

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THE ACTION OF HEPARIN, SERUM ALBUMIN (CRYSTALLINE), AND SALMINE ON BLOOD-CLOTTING MECHANISMS (IN VITRO)

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The two phases of blood-clotting, viz. 1, formation of thrombin from prothrombin, and 2, thrombic conversion of fibrinogen into fibrin, may be studied quantitatively *in vitro* (1). A rational approach to the experimental analysis of the mode of action of any anticoagulant is to observe its behavior in the respective phases of such artificially isolated systems. This type of approach led Howell (2) to separate clot-inhibitors into *a, antiprothrombic,* if they act on the first phase, and *b, antithrombic,* if the action involves the second phase. We shall adhere to these general terms and, again following Howell, shall designate as *thromboplastic* any agent which assists the first phase reaction. The term *fibrinoplastic* will be used for any assistance in the second phase.

Since serum albumin and salmine (a basic protamine, which combines with heparin (3), after the manner of heparin-protein compounds (4)) have been reported to be anticoagulants, each showing some interesting relationships to the action of heparin, the following investigation was undertaken in an attempt to analyse the fundamental mechanisms involved.

Experimental technique. Methods for preparing the various clotting agents (prothrombin, cephalin, $CaCl_2$, thrombin, fibrinogen) have previously been detailed (1), together with the technique for the clotting-tests and for the study of the first phase (prothrombin activation). The crude *thromboplastin* used for most of the present experiments consisted of an aqueous extract (diluted) of fresh or frozen dog brain or of the acetone-precipitated, air-dried residue of similar extracts. *Crude plasma "albumin"* consists merely in the dialysed plasma residue after removal of fibrinogen and globulins by half-saturation with $(NH_4)_2SO_4$.

We are indebted to 1, Dr. C. H. Best and the Connaught Laboratories (Toronto) for a purified lung-*heparin,* assaying 110 "units" per mgm.; 2, Dr. T. L. McMeekin (Harvard) for the *crystalline serum albumin*; 3, Messrs. E. R. Squibb and Sons (Brunswick, N. J.) for the *salmine* (*-sulfate*), and 4, Dr. J. H. Northrop (Rockefeller Institute) for the *crystalline trypsin.*[1]

pH was controlled (unless otherwise stated) with the glass electrode (Beckman pH meter), routinely to pH = 7.25. The heparin (1:1000 soln.) had an initial pH

[1] *Crystalline trypsin* is now available commercially (Plaut Research Laboratories, Lehn & Fink Products Corp., Bloomfield, N. J.).

of 4.1, well buffered; the salmine sulfate (1:200 soln.) was very poorly buffered at pH = 5.1; a 1:10 dilution of McMeekin's crystalline serum albumin (before dialysis) gave a pH of 5.85, also poorly buffered.

Volumetric pipettes were used for accurate measurement of the quantities of the various reagents and all clotting-tests (2nd phase) refer to time of appearance of first visible strands of fibrin in mixtures held in small tubes in a thermostat water-bath at 38°C.

Plasma experiments. Since the action of the cited anticoagulants on whole blood and plasma has been studied repeatedly by a number of investigators, the data of table 1 serve principally to show modifications of the inhibitory phenomena in the presence of various thromboplastic agents (cephalin: crystalline trypsin). The ability of trypsin (thrombo-

TABLE 1

Effects of heparin and salmine on clotting of recalcified citrated dog plasma, in presence of various thromboplastic agents

Clotting-times (seconds), at 38°C., for 1 cc. plasma + 0.25 cc. N/10 $CaCl_2$ + 0.75 cc. of thromboplastic and other agents (quantities expressed in *milligrams*).

NUMBER	INHIBITOR	(*Ca*, ONLY)	CEPHALIN (0.5)	XTAL. TRYPSIN (0.05)	TRYPSIN (0.05) + CEPHALIN (0.5)
1		140 sec.	45 sec.	110 sec.	15 sec.
2	Heparin (0.1)	510 sec.	240 sec.	270 sec.	20 sec.
3	Heparin (0.5)	∞	∞	∞	290 sec.
4	Salmine (0.025)	400 sec.	100 sec.	120 sec.	15 sec.
5	Salmine (0.25)	1040 sec.	180 sec.	240 sec.	25 sec.
6*	Salmine (2.5)	>1 hr.	ē 40 min.		
7	Hep. (0.1) + Salm. (0.025)	240 sec.	60 sec.	185 sec.	16 sec.
8	Hep. (0.5) + Salm. (0.025)	∞	310 sec.		
9	Hep. (0.5) + Salm. (0.25)	270 sec.	105 sec.	∞	22 sec.
10*	Hep. (0.5) + Salm. (2.5)	>6<16 hrs.	>2<6 hrs.		

* Flocculated.

plastic enzyme), especially in conjunction with added cephalin, to overcome most of the inhibitory effect of heparin (5) is again demonstrated. The same effect is shown also for salmine and, even more strikingly, for salmine *plus* heparin. pH was not controlled in these experiments, but plasma is well-buffered.

The heparin-salmine antagonism (3) is confirmed.

Serum albumin experiments. Tests with crude plasma "albumin." 1. *First phase* reactions were not studied with this material. 2. *Second phase* studies confirmed and extended the observations previously reported (5). There was usually an insignificant retardation of the clotting of fibrinogen by thrombin, in the presence of the "albumin" preparation, even when pH was controlled (7.25). Sometimes, however, a slight

fibrinoplastic effect (clot-acceleration) occurred instead, especially with the more dilute preparations. Table 2, no. 2, is typical of several experiments designed to test Quick's (6) assertion of a "progressive" antithrombic effect. On incubating a *stable* thrombin (no. 1) for an hour at room temperature (24°C.) with the "albumin" preparation, there was seen only a minor and irregular effect on thrombic activity (tested at the cited intervals), not at all like the marked "progressive" inactivation described by Quick.

As before (5), however, the striking synergism with heparin (Quick) is again manifested (no. 4).

Tests with crystalline serum albumin. 1. In the *first phase:* During the formation of thrombin from prothrombin, added crystalline serum albumin was consistently *thromboplastic* throughout a number of experiments of the type illustrated in table 3. Furthermore, it always lessened the

TABLE 2

Effects of incubation (with thrombin) on the antithrombic action of crude plasma "albumin" and heparin (1:5000)

Thrombin (1 volume) + anticoagulant (½ volume, each, suitably diluted): incubated, at 24°C., for times (minutes) indicated. Clotting-times (seconds), at 38°C., for 1 cc. fibrinogen + 0.5 cc. mixture.

NUMBER		¼ MINUTE	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
1	(Control)	18 sec.	18 sec.	18 sec.	18 sec.	18 sec.
2	Albumin	18 sec.	10 sec.	12 sec.	16 sec.	33 sec.
3	Heparin	25 sec.	25 sec.	25 sec.	25 sec.	25 sec.
4	Alb. + Hep.	39 min.	>4 hr.	>4 hr.	>4 hr.	>4 hr.

antiprothrombic action of heparin, in parallel tests, whether a weak brain thromboplastin or cephalin was the main thromboplastic agent used.

2. In the *second phase*: The "immediate" effect of adding crystalline albumin to fibrinogen-thrombin mixtures showed the same minor variability on clotting-time as in the case of the corresponding experiments with crude "albumin." Thus, there was a few seconds' lengthening of the clotting-time in most instances, but an occasional slight acceleration occurred in some experiments.

Varying the salt content by dialysis (as compared with simple dilution) or by the use of 0.85 per cent NaCl (*vs.* distilled water) for several dilutions, gave no indication of any relationship of these effects to the salt content. Simple pH effects were ruled out by having the albumin, thrombin, and fibrinogen all at the same pH (7.25). Controlled modification of pH, in both first and second phase reactions, will be discussed in a separate section.

TABLE 3

Effects of crystalline serum albumin and heparin on the activation of prothrombin to thrombin

Thrombic mixture (T) = 4.0 cc. prothrombin + 0.25 cc. N/10 $CaCl_2$ + 0.75 cc. thromboplastic agent (A, dilute brain extract; B, cephalin) *plus* anticoagulant, incubated, at 10°C., for time (minutes) indicated. Clotting-times (seconds) for 1 cc. fibrinogen + 0.5 cc. T, at 38°C. pH = 7.25.

NUMBER		5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES	120 MINUTES
		sec.	*sec.*	*sec.*	*sec.*	*sec.*
	A. Brain thromboplastin					
1	(Control)	40	15	12	12	12
2	Crystalline albumin	18	15	15	17	18
3	Heparin	262	72	29	21	19
4	Albumin + heparin	200	33	24	21	21
	B. Cephalin (weak)					
5	(Control)	145	115	85	43	
6	Crystalline albumin	85	45	25	15	
7	Heparin	2700	2370	1860	960	
8	Albumin + heparin	1530	1580	1320	750	

TABLE 4

Effects of incubating thrombin with crystalline serum albumin (McMeekin's, diluted 1:20) and heparin (1:1000)

Clotting-times (seconds) at 38°C., and pH = 7.1, for 1 cc. fibrinogen + 0.5 cc. anticoagulant *plus* thrombin mixtures. Mixtures, consisting of 1 volume thrombin solution + 1 volume of anticoagulant (diluted, where necessary), held at 24°C. and pH = 7.1, for times (minutes) indicated.

NUMBER		¼ MINUTE	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
		sec.	*sec.*	*sec.*	*sec.*	*sec.*
	A. Stable thrombin:					
1	(Control)	18	18	18	18	18
2	Albumin*					
3	Heparin (1:1000)	24	26	26	26	26
4	Alb. + Hep.	27	42	42	42	42
	B. Slightly unstable thrombin:					
5	(Control)	14	14	14	15	18
6	Albumin (1:20)	21	22	25	29	36
7	Heparin (1:1000)	48	78	115	160	185
8	Alb. + Hep.	73	103	138	165	205

* Tests with albumin alone are lacking in series A, but a test made at the same time, on another thrombin, showed the usual *slight* (few seconds) initial "antithrombic" effect, and in some other tests this showed no increase in 1 hr.

Incubation of thrombin with crystalline serum albumin for an hour gave no evidence of any significant "progressive" effect.

In sharp contrast to the crude "albumin," there was *no* sharp increase in antithrombic effect with heparin *plus* the pure albumin. In cases where the albumin was slightly antithrombic, there was a minor additive effect superimposed on the similar heparin action. When the albumin was fibrinoplastic, the heparin effect was reduced. Besides being of small magnitude, the aforementioned antithrombic addition effect was *non-progressive* in the case of stable thrombins (4, no. 4).

Effect of stability of thrombins. Some thrombin preparations are much less stable than others, even when held at low temperatures (10°–15°C.). We have observed that a thrombin, which tends to undergo spontaneous inactivation, even if the rate of deterioration is so slow that it barely shows up within the hour period of the accompanying tests (see control, no. 5), does give progressively longer clotting-times in the presence not only of albumin, but also with heparin alone and, most markedly, with heparin *plus* albumin. Obviously, this is not a synergism of the albumin and heparin effects, but a modification of the thrombin inactivation process. The inactivation phenomenon will be the subject of a separate communication (7).

Experiments with salmine. 1. In the *first phase*: The presence of salmine-sulfate (at same pH as the other reagents) resulted in a marked *antiprothrombic* effect in the early phases of prothrombin activation. This was found consistently, except in a few cases (upon which we place no emphasis) where the thromboplastic agent was omitted. As usual in these cases, the activation by calcium alone was slow and poor. The anomalous thromboplastic action of salmine was noted in the early phases (in some experiments even more conspicuously than in the example cited). In most cases, the antiprothrombic effect developed later, although rather irregularly, as in the cited experiment (no. 8).

Heparin (v. infra) is also an antiprothrombin, but the data of table 5 clearly reveal the *antagonism* between salmine and heparin in this respect. With the right quantitative mixture (no. 6), the antiprothrombic effects of the two agents neutralize each other completely and the activation curve accurately reproduces that of the control (no. 1).

2. In the *second phase*: Salmine was consistently fibrinoplastic in the thrombin-fibrinogen reaction and sometimes quite strikingly so. The action was quantitative, disappearing on sufficient dilution. It was non-progressive. These facts are brought out in table 6.

The relations of salmine and heparin effects in the second phase are shown in table 8, in the section dealing with pH effects. It is seen, throughout the pH range, that salmine *immediately* overcomes most of the minor direct antithrombic action of heparin.

Experiments with heparin. The data on the unequivocal *antiprothrombic* action of heparin in the first phase and the very minor immediate (non-

progressive) *antithrombic* effect in the second phase (at all pH levels tested) are included in the accompanying tables. The experimental results also show that the antiprothrombic action of heparin is neutralized in a quantitative manner by salmine, but is only slightly reduced by crystalline serum albumin. In the second phase, the minor antithrombic

TABLE 5

Effects of salmine (-sulfate) and heparin on activation of prothrombin to thrombin

Thrombic mixture (T) = 4 cc. Prothrombin + 0.25 cc. dil. brain extract (nos. 1–6 only) + 0.25 cc. N/10 $CaCl_2$ + inhibitors (quantities expressed in *milligrams*) to total volume = 5 cc. Incubation (at 25°C.) for times indicated (minutes). Clotting-times (seconds) for 1 cc. fibrinogen + 0.5 cc. T, at 38°C. pH = 7.25.

NUMBER		2 MINUTES	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
		sec.	*sec.*	*sec.*	*sec.*	*sec.*
1	(*Ca* + brain extr.)	7	5	5	6	8
2	Salmine (0.25)	3060	360	20	7	7
3	Heparin (0.05)	9	6	6	8	8
4	Heparin (0.25)	120	16	12	11	11
5	Salm. (0.25) + Hep. (0.05)	280	90	11	7	9
6	Salm. (0.25) + Hep. (0.25)	7	5	5	6	8
7	(*Ca*, alone)		1800	1800	900	240
8	Salmine (0.25)		695	1920	1500	1320

TABLE 6

Effects of varying amounts of salmine (-sulfate) incubated with thrombin, to test action on the second phase of clotting

Thrombic mixture (T) = 2 volumes thrombin + 1 volume salmine (cited strengths), incubated, at 24°C., for times (minutes) indicated. Clotting-times (seconds), at 38°C., for 1 cc. fibrinogen + 0.5 cc. T. pH = 7.25.

NUMBER		¼ MINUTE	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
		sec.	*sec.*	*sec.*	*sec.*	*sec.*
1	(Control)	22	21	22	22	26
2	Salmine (0.02 per cent)	20	19	20	20	32*
3	Salmine (0.1 per cent)	18	17	18	18	20
4	Salmine (0.5 per cent)	14	14	14	14	15

* Flocculation in mixture.

action of heparin is largely overcome by salmine, but is usually slightly increased by albumin.

When there is some inactivating process (7) in the thrombin preparation itself, this action (table 4) seems to be speeded up by heparin or albumin or both (additively). The "progressive" effect (cf. 6) is that of the throm-

bin-inactivator (*progressive* antithrombin (7)) and not of the heparin or albumin per se.

Fisher (8), in experiments on "genuine" (native) hen plasma, alleged a slight acceleration of clotting (in presence of tissue extract) when very small concentrations of heparin were added. This we have been totally unable to confirm with our reagents.

e.g. Using same reagents and test conditions as in data of table 7, plasma (dog, citrated) was clotted (by Ca + thromboplastin) in the presence of a series of heparin dilutions. The anticoagulant effect diminished steadily down to the absence of action finally obtained, as seen in the last four tests, *viz.*

heparin (mgm. *per test*) = $\frac{1}{160}$, $\frac{1}{320}$, $\frac{1}{640}$, $\frac{1}{1280}$;
respective clotting-times = 19, 18, 17, 17 sec.:
control (with dist. water) = 17 sec.

TABLE 7

Effect of pH on clotting of plasma, with and without heparin (1:1000) and salmine (1:100)

Samples of citrated, Berkefelded, dog plasma, diluted with an equal volume of dist. water, were brought to pH values cited. Clotting-times (seconds) at 38°C. for 1 cc. plasma + 0.25 cc. anticoagulant + 0.25 dilute brain thromboplastin + 0.25 cc. N/8 $CaCl_2$.

pH	CONTROL	HEPARIN	SALMINE (SECONDS)	CONTROL
	sec.		*flocc.*-agglut.*	*sec.*
6.0	47	Flocc. 23½ min.	60– 90	47
6.5	22	Flocc. clot 4½ min.	45– 60	21
7.0	19	130 sec.	40– 50	17
7.5	16 (16)	100 (98) sec.	35– 47	15½
8.0	15	78 sec.	40– 50	14
9.0	17 (17)	56 (57) sec.	45– 65	15
10.0	50	165 sec.	100–200	48

* Flocculation preceded agglutination in the salmine experiments: the latter is the closer approximation to a true clot.

The above-described antiprothrombic and antithrombic effects of heparin in the isolated clotting systems also diminish steadily to a vanishing point with successive dilutions of the heparin. Concentrations which have lost the ability to affect the second phase are still strongly antiprothrombic in the first phase, if cephalin (or very weak thromboplastin) is used (9).

Effects of pH. A. *On clotting of whole plasma.* In the data of table 7, the clotting-times (38°C.) of recalcified citrated plasma (with added thromboplastin) are studied at various pH levels in the range 6.0–10.0. The effects of heparin and salmine are also included. Both are inhibitory throughout the pH range tested. In the controls the optimum pH is definitely on the alkaline side of neutrality, in the neighborhood of pH = 8.0. The anticoagulant action of heparin is minimal at pH = 9.0. The

sharp increase in its inhibitory action below pH = 6.5 is accompanied by flocculation. Salmine produces a flocculation which develops in the plasma at all pH levels approximately at the times noted. With the onset of clotting the flocculation becomes coarse and agglutinative and only an approximate timing is possible. There is a suggestion that the pH optimum shifts slightly toward the acid, but the data are not sufficiently reliable to establish the optimum within 0.5 to 1.0 pH unit. The salmine tests were performed several hours later than the heparin series and the controls have altered very slightly in the direction of a somewhat more rapid clotting towards the alkaline side. This supports a finding which we have frequently experienced, both with plasma and pro-

TABLE 8

Effect of pH on clotting of fibrinogen by thrombin, in presence of crystalline serum albumin, salmine and heparin

Clotting time (seconds) at 38°C. and cited pH values, for 1.0 cc. fibrinogen + 0.5 cc. anticoagulant + 0.5 cc. thrombin. Amount of anticoagulant in each clotting test is expressed in milligrams, except crystalline albumin, which is measured in cubic centimeters of a 1:20 dilution (not dialysed) of the turbid suspension supplied by Dr. T. L. McMeekin. The last two experiments were made several hours after the first four series.

NUMBER		10.0	9.0	8.0	7.0	7.0	6.0
			sec.	sec.	sec.	sec.	
1	(Control)	Trace, 4 hours	15	10	12	15	110 sec.
2	Albumin (0.25)	No clot	20	15	16	21	360 sec.
3	Salmine (0.25)	9 sec.	3	6	9	12	40 sec.
4	Heparin (0.25)	Trace, 6 hours	30	25	46	60	Turbid (no clot)
5	Hep. (0.25) + Alb. (0.25)	No clot	43	33	50	75	Fine flocc. (10 min.)
6	Hep. (0.25) + Salm. (0.25)	Trace, 6 hours	26	20	19	30	70 sec.

thrombic systems, viz., that thrombic activity may be enhanced after a few hours' keeping.

B. *In the second phase.* In the tests of table 8, the individual reagents were brought separately to each pH value and rapidly mixed and held at 38°C. The controls show a definite optimum, again close to pH = 8.0 although it is not defined by these experiments more closely than ±1 pH unit. There is a falling off of thrombic activity toward both the alkaline and the acid side of the optimum.

The *fibrinoplastic* action of salmine (no. 3) is shown throughout the pH range (6.0–10.0) and is most striking at the highly alkaline pH = 10, where clotting is all but inhibited in the control (and other) tests. In the data cited, the pH optimum is shifted by salmine some 1–2 pH units toward the alkaline side.

The minor *antithrombic* action of crystalline serum albumin has the same pH optimum as the control.

The similar weak *antithrombic* action of heparin also adheres to the same optimum, and this is true also of the heparin *plus* albumin. There is apparently a slight shift to the acid of the pH optimum in the case of heparin + salmine. There is no doubt that heparin has overcome the tendency of salmine to shift the optimum pH toward the alkaline side. With the reciprocal neutralization of the heparin and salmine effects, the normal (control) clotting-times are restored (approximately).

C. *In the first phase.*

Conditions for studying the effects of pH on antiprothrombic reactions are undoubtedly complicated by effects of pH (alone) on the prothrombin → thrombin reaction as well as by an influence on thrombin destruction which, at least in the later phases, seems to go on simultaneously with thrombin formation at the more acid pH levels. The method of study consists in bringing the prothrombin (alone, and with added anticoagulant) to the desired pH, then adding the thromboplastic agent and $CaCl_2$ (which tests showed to be without effect on the pH of the mixture) and incubating, at low temperature, in the usual way. The actual clotting test is performed at 38°C. by adding 0.5 cc. of thrombic mixture to 1.0 cc. fibrinogen, buffered with 1 per cent sod. barbital (at pH = 7.0 in the experiments cited in table 9, but at a somewhat more alkaline pH in other tests, confirmatory of these data). Controls at each pH are to be compared with the activation curves in the presence of the antiprothrombic agent. Preliminary tests proved that the thrombic mixture (sans Ca) at pH = 10 shifted the pH of the buffered fibrinogen less than 0.2 pH unit (to alkaline side) while the mixture at pH = 5.0 produced a shift of less than 0.35 pH unit (to acid side). These negligible changes ensure that the pH variations in the thrombic mixtures are without influence of the type studied in the preceding section.

Since the prothrombin and salmine solutions were salt-poor and feebly buffered, further control tests were made on the actual thrombic mixtures to see if the pH values were maintained. At the extremes, there was only 0.1–0.2 pH unit shift in 30–40 min. and 0.5–0.9 pH unit in 1½ hours. This was considered very satisfactory.

Since crystalline serum albumin was thromboplastic in the first phase reaction, it was not included in this study of pH in relation to antiprothrombic action.

Controls. With our particular reagents, the controls show a pH optimum surprisingly far into the alkaline region (*cf.* 16). Although the data of these and some other similar experiments define the optimum only within 1 pH unit, it is repeatedly demonstrated that the optimum is in the zone, pH = 8–9. There was a slight inhibition on increasing the alkalinity to pH = 10.0 and a much more striking inhibition in the acid region (pH = 5.0–6.0), the latter apparently associated with a heightened thrombin destruction.

Salmine. Two separate experiments, on different occasions, are included in table 9. In both cases brain thromboplastin was used as the

activator (with Ca) of the prothrombin. Comparison of the controls (*a*) and salmine (*b*) in experiment A shows that the antiprothrombic action of the salmine was exhibited, especially in the early phases, throughout the pH range 6.0–10.0. Experiments at pH 8.0 and 10.0 were also performed on the second occasion, but are not included in series B since the results were similar to the foregoing. The data of experiment B at pH = 6.0 and 5.0 give evidence of an *initial thromboplastic action* instead. In no. 5*a* this was followed by the usual inhibitory effect, but in no. 6*a* this did not develop within the time limit (1 hr.) of the tests.

TABLE 9

Effect of pH on activation of prothrombin, with and without salmine

Clotting-times (seconds), at 38°C., for 1 cc. fibrinogen + 0.5 cc. sod. barbital buffer (pH = 7.0) + 0.5 cc. thrombic mixtures (T). T-mixtures = 4 cc. prothrombin + 0.5 cc. 1:1000 soln. salmine sulfate (or distilled water) + 0.25 cc. dil. brain extract + 0.25 cc. N/10 $CaCl_2$, incubated at 10°C. for times indicated (minutes).

	pH	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES
		sec.	*sec.*	*sec.*	*sec.*
A					
1a (Salmine)	10.0	220	110	60	20
1b (Control)	10.0	20	5	4	4½
2a (Salmine)	9.0	155	65	25	8
2b (Control)	9.0	6	4	4	4½
3a (Salmine)*	7.0	220	110	40	12
3b (Control)	7.0	35	16	10	5
4a (Salmine)*	6.0	275	295	315	400
4b (Control)*	6.0	133	130	87	65
B					
5a (Salmine)*	6.0	60	90	450	375
5b (Control)*	6.0	220	220	210	115
6a (Salmine)*	5.0	60	100	225	240
6b (Control)*	5.0	1140	1440	2040	1500

* Flocculation.

Heparin. Table 10 illustrates a similar study with heparin. Here again, the antiprothrombic action is manifest throughout the pH range (5.0–9.0). At no time was heparin thromboplastic. The sharp rise in anticoagulant effect noted in the last two readings at the acid values (pH = 5.0–6.0) was not progressive between 60 and 90 minutes and suggests an antithrombic rather than an antiprothrombic action. The controls show a similar, though much less marked, thrombin instability.

DISCUSSION. The present data, selected from a considerable number of similar experiments, re-affirm the stand we have previously taken (5), in support of the original view of Howell and Holt (10), viz., that heparin

is indeed an "antiprothrombin," in the general sense of being an agent which retards or prevents conversion of prothrombin to thrombin. In emphasizing the importance of the *type* of thromboplastic agent as determining the degree and duration of the antiprothrombic action of heparin, it was pointed out (11) that failure to realize this was responsible for a number of current denials of the antiprothrombic effect. We would add that it is not necessary to postulate an additional plasma factor (*cf.* 12) for the heparin inhibition of thrombin formation in the presence of tissue thromboplastin, provided that the action is looked for early enough and a sufficiently weak thromboplastin is used. The accompanying data stress these points, particularly the quantitative aspects of heparin-thromboplastin interrelationships. A strong tissue extract overcomes the heparin

TABLE 10

Effect of pH on activation of prothrombin, with and without heparin

T mixtures = 4 cc. prothrombin + 0.5 cc. heparin (1:2000) (or distilled water) + 0.25 cc. dilute brain extract + 0.25 cc. N/10 $CaCl_2$, held at respective pH values, and low temperature (10°C.) for times (minutes) indicated. Clotting-times (seconds), at 38°C., for 1.0 cc. fibrinogen + 0.5 cc. sod. barbital (1 per cent), buffered at pH = 7.0, + 0.5 cc. T.

	pH	5 MINUTES	15 MINUTES	30 MINUTES	60 MINUTES	90 MINUTES
		sec.	*sec.*	*sec.*	*sec.*	*sec.*
1a (Heparin)	9.0	107	42	50	55	
1b (Control)	9.0	12	10	10	11	
2a (Heparin)	7.0	257	83	45	36	
2b (Control)	7.0	42	17	12	11	
3a (Heparin)	6.0	535	275	185	1020	960
3b (Control)	6.0	90	50	70	57	47
4a (Heparin)	5.0	700	600	470	1000	960
4b (Control)	5.0	200	125	120	220	212

inhibition in all but the first minute or two of the prothrombin activation: a weak (brain) thromboplastin, like cephalin, is ineffective, or retarded over a period of an hour or more, if sufficient heparin is used.

The experimental data do not distinguish between two underlying possibilities, viz. 1, a direct action of heparin on prothrombin ("antiprothrombic" in a strictly literal sense), and 2, some process of "neutralization" of the thromboplastic factor(s) by heparin ("antithromboplastic" or "antithrombokinase" action in the sense of several previous workers). There is a definite dearth of evidence favoring any direct relationships.

SUMMARY

An experimental analysis has been made of the effects of serum albumin, salmine, and heparin on the isolated first and second phases of blood-clotting reactions. Acid-base influences have been investigated.

Crystalline serum albumin has a nonspecific thromboplastic action in the prothrombin → thrombin phase and a slight effect (usually antithrombic) in the thrombin + fibrinogen interaction. These phenomena are unimportant in relation to natural coagulation mechanisms. The purified albumin *lacks* the ability, consistently present in crude plasma "albumin" preparations, of producing a marked synergistic antithrombic action, in conjunction with heparin.

Salmine is antiprothrombic in the first phase and fibrinoplastic in the second. The inhibition preponderates in plasma. Both actions are antagonized by heparin.

Heparin is antiprothrombic in the first phase, to a degree and duration depending on quantitative relations to the thromboplastic factors. In the second phase, it has a minor immediate (nonprogressive) antithrombic effect, exaggerated (usually) by albumin and antagonized by salmine.

The significant anticoagulant effects in these studies are the *antiprothrombic* actions of heparin and salmine. There is some evidence that the minor immediate antithrombic actions of heparin and albumin (especially additively) favor the natural progressive thrombin inactivator (i.e., antithrombin, in the sense of serum "antithrombin") but the so-called proantithrombic (antithrombinogenic) factor of plasma and serum has not been considered a part of these studies.

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THE RATE OF RESPIRATORY ADJUSMENT TO POSTURAL CHANGE[1]

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Most of the errors in measurement of respiratory functions caused by physiologic factors tend to result in values that are too high. These errors may be caused by conditions such as an insufficient period of rest, too recent ingestion of food, breathing against a resistance, or discomfort from the face mask. In view of the discrepancies between the basal respiratory volumes found by us (1) and those found previously by many other investigators, it seemed desirable to ascertain the factors responsible. One of the obvious technical differences was the greater length of the rest period in our experiments as compared with the rest period in experiments of others. While it is generally recognized that subjects respire less air per unit of time after a period of rest, we have been unable to find any information in the literature as to the rate or the extent of this decline in respiratory volume. In order to assess the increment in respiratory volume caused by breathing carbon dioxide, these increments must be measured from a baseline, and the establishment of this baseline is impossible unless the length of the required rest period is known. Most experimenters have felt that practice affects the results since any subject going through an unfamiliar test is likely to have some emotional response. If this be true, one would expect that respiratory volumes would decrease with repeated testing and that the stabilized level might be reached sooner.

The data given in this report allow us to determine the length of the necessary rest period for establishing stable respiratory volumes in young adult males, the effect of training on the rate of stabilization of respiratory volumes and the individual variations in the rate at which respiratory volumes are stabilized.

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For statistical advice and criticism of the manuscript we are indebted to Dr. Robert C. Tryon and Dr. Herbert S. Conrad.

Grateful acknowledgment is made for clerical assistance in the analysis of the data to the Works Progress Administration (O. P. No. 65-1-08-62 Unit A 8).

EXPERIMENTAL. A group of 31 white, male college students (N. Y. A.) from 18 to 24 years of age served as subjects. Each subject was tested at a uniform time of day (either in the morning or in the afternoon) and had not eaten for one hour prior to each test. A Siebe-Gorman half-mask was securely tied over the subject's face while he was standing, and immediately after he had assumed the supine position the collection of the expired air was begun. He breathed outdoor air warmed to room temperature. Inspired and expired air were separated by a pair of egg-shell valves floating on mercury which required a pressure of only 1.5 mm. of water in order to open. The expired air was collected in a pair of recording spirometers of the Tissot type, each with a capacity of 9.19 liters (at 0°C., 760 mm. Hg). The apparatus was arranged so that time was recorded automatically at the end of expiration of each 9.19 liter sample of air. Continuous samples of expired air were measured over a period of 30 to 40 minutes. Respiratory volumes in liters per minute were computed from the time required to breathe out each 9.19 liter sample and were corrected to 0°C. and 760 mm. mercury. Each subject served for four experimental periods on different days.

TREATMENT OF DATA. Because of the individual differences in respiratory volumes, interpolated values were used in the calculation of average curves. Estimates were made of the respiratory volumes at minute intervals in each experiment from curves in which total elapsed time was plotted as the ordinate and successive 9.19 liter units of air were plotted as the abscissa. The respiratory volumes in liters were thus obtained for each minute from one to thirty, and were corrected for the size of the subject by dividing the recorded volumes by the surface area as estimated from the height and weight according to the DuBois formula. Zero time was taken at the moment the subject assumed the supine position. The respiratory volume of each of the 31 subjects appeared in every point in all four tests. Values were obtained for 30 minutes in the first three tests and for 20 minutes in the fourth test.

The results of the experiments are shown in table 1 and figure 1.

A rapid decrement in the respiratory volume occurred with the assumption of the supine position and an average of 20 minutes was required before this decrement became insignificant. Although a decrease was apparent between 20 and 30 minutes, it was not statistically significant.

A comparison of the four curves indicates that practice does not significantly alter the rate at which the respiratory volume becomes stabilized. The decrement must therefore be the result of rest plus the physiologic adjustment to postural change rather than to psychological factors related to unfamiliarity with the experimental technique. It should also be noted that the actual respiratory volumes do not decrease as more tests are done—an indication that with adequate preliminary rest periods extended training is not necessary.

Although no effects of practice were discernible in the average curves for the entire group, it seemed possible that some subjects would attain stable respiratory volumes more quickly in later experiments than on first

TABLE 1

Average respiratory volume for successive minutes after assuming supine posture

N = 31 adult males

MINUTES OF REST	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV	
	Mn.†	$\sigma_{Mn.}$†	Mn.†	$\sigma_{Mn.}$†	Mn.†	$\sigma_{Mn.}$†	Mn.†	$\sigma_{Mn.}$†
1	5.08	0.22	5.20	0.24	5.08	0.23	5.29	0.22
2	4.56	0.20	4.47	0.17	4.57	0.18	4.57	0.18
3	4.27	0.17	4.19	0.14	4.23	0.14	4.33	0.17
4	4.05	0.15	3.99	0.13	4.06	0.14	4.11	0.16
5	3.97	0.14	3.87	0.13	3.92	0.13	3.97	0.15
6	3.85	0.14	3.79	0.13	3.81	0.12	3.88	0.14
7	3.74	0.13	3.71	0.13	3.73	0.12	3.75	0.14
8	3.69	0.14	3.69	0.13	3.73	0.13	3.72	0.14
9	3.62	0.12	3.64	0.13	3.71	0.14	3.65	0.13
10	3.56	0.12	3.59	0.14	3.65	0.14	3.57	0.13
11	3.50	0.11	3.57	0.13	3.60	0.13	3.56	0.12
12	3.46	0.11	3.56	0.13	3.57	0.13	3.53	0.12
13	3.46	0.11	3.53	0.12	3.57	0.13	3.52	0.12
14	3.45	0.11	3.49	0.11	3.58	0.13	3.52	0.11
15	3.46	0.11	3.46	0.10	3.54	0.11	3.49	0.11
16	3.48	0.11	3.44	0.10	3.53	0.10	3.49	0.11
17	3.54	0.13	3.46	0.10	3.46	0.09	3.47	0.12
18	3.55	0.15	3.43	0.09	3.43	0.10	3.45	0.11
19	3.48	0.14	3.36	0.09	3.42	0.08	3.41	0.11
20	3.49	0.14	3.39	0.09	3.42	0.08	3.44	0.11
21	3.42	0.12	3.34	0.09	3.39	0.09	*	*
22	3.44	0.12	3.37	0.09	3.43	0.09		
23	3.41	0.13	3.38	0.09	3.47	0.10		
24	3.41	0.13	3.39	0.09	3.52	0.13		
25	3.39	0.12	3.34	0.09	3.51	0.12		
26	3.37	0.11	3.34	0.09	3.47	0.10		
27	3.33	0.11	3.36	0.09	3.42	0.09		
28	3.29	0.11	3.34	0.09	3.40	0.08		
29	3.31	0.11	3.34	0.10	3.43	0.09		
30	3.36	0.11	3.37	0.10	3.43	0.09		

† Liters per square meter per minute.

* Experiment IV terminated at 20 minutes.

trial. To test this possibility, the time required for each subject to stabilize his respiratory volume in each test was determined. The average of these estimations as determined independently by two observers was used to plot the frequency distributions as shown in figure 2. The results con-

firmed the findings for the average curves for all subjects; the mean time for the first test run was 20.6 ± 0.62 minutes, for the second run 19.25 ± 0.54 minutes and for the third run 19.01 ± 0.59 minutes. Inspection of the data from which figure 3 was derived showed that 6 of the 30 subjects required less time in runs 2 and 3 to stabilize their respiratory volumes

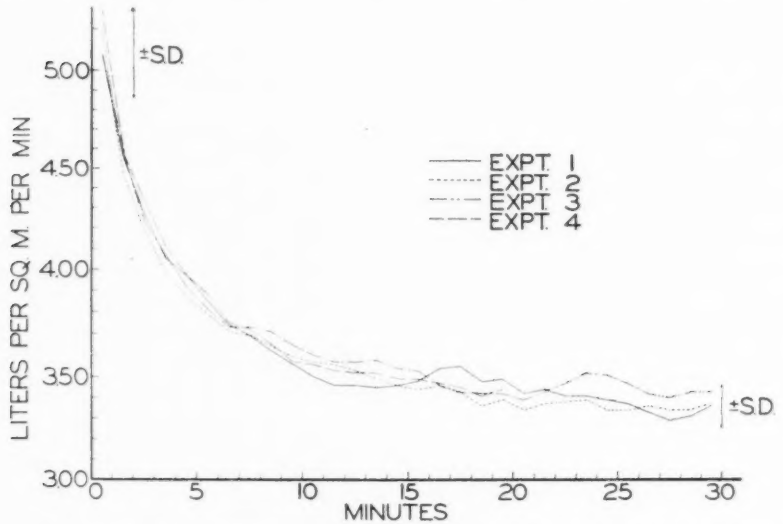

Fig. 1. Average decrement in respiratory volume after assuming supine posture. Average curves for 31 males – ages 18 to 24. Mn 23.16 years. Results from 4 experiments shows absence of learning effects. Length of arrow at beginning and end of curves shows ± 1 S. D. of the mean values. Zero time taken at moment of lying down. Mean values plotted at middle of temporal interval.

TABLE 2

Mean decrement in respiratory volume at 10-minute intervals after assuming supine position

N = 31

EXPERIMENT	MINUTE 1-10			MINUTE 10-20			MINUTE 20-30		
	Mn. l./sq.m./min.	σ_{Mn}	C.R.†	Mn. l./sq.m./min.	σ_{Mn}	C.R.†	Mn. l./sq.m./min.	σ_{Mn}	C.R.†
I	1.52	0.18	8.2	0.07	0.10	0.7	0.13	0.05	2.4
II	1.61	0.19	8.6	0.20	0.07	2.5	0.02	0.03	0.7
III	1.44	0.17	8.3	0.22	0.07	3.1	0.01	0.04	0.3
IV	1.71	0.20	8.4	0.14	0.03	4.3	*	*	*

* Experiment IV terminated at 20 minutes.

† Critical ratio.

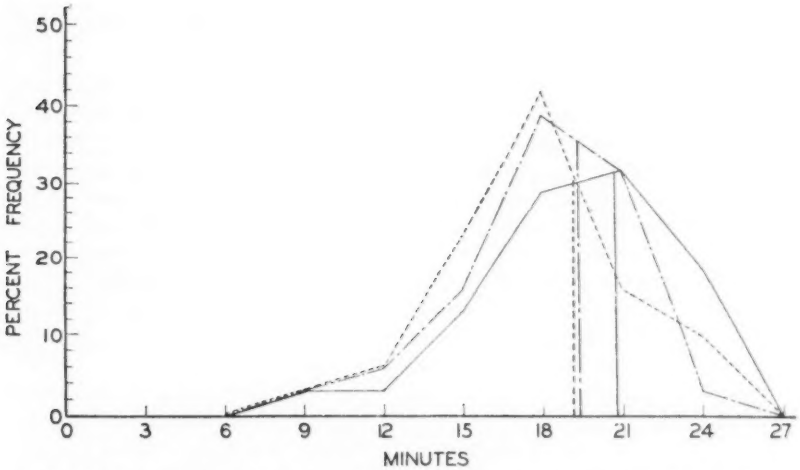

Fig. 2. Frequence distributions of the time required by individual subjects to attain stable values of respiratory volume.

—— test I; — — — test II; . . . . test III. Mean values shown by vertical lines. The same 31 subjects were used in each of the 3 tests.

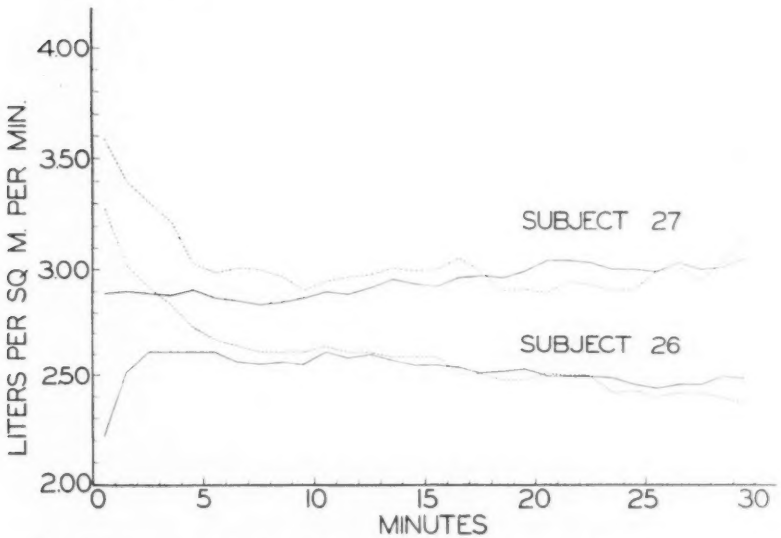

Fig. 3. Effect of postural change on decrement in respiratory volume.

—— Respiratory volume in individual subject after 30 minutes of rest in supine position. Zero time at 31 minutes after assuming supine posture.

. . . . Respiratory volume without previous rest. Zero time at moment of assuming supine posture.

Experiments on different days.

but that reversals of this effect in some of the other subjects were sufficient to establish the trend as shown in figures 1 and 2.

Table 2 shows that the decrement in respiratory volume is significant from the tenth to the twentieth minute but insignificant from the twentieth to the thirtieth minute.

In order to determine just what the effects of tying on the face mask would be, 3 subjects were put through the following experiment. Each subject rested for 30 minutes in the supine position. Then the Siebe-Gorman half-mask was tied to his face and the respiratory volumes were recorded as soon as the mask had been adjusted. The results (fig. 3) indicate that the respiratory adjustment was made during the 30-minute rest period and respiratory volume was not significantly increased by the application of the mask.

Discussion. The results of this experiment show that a 20-minute rest period in the supine position suffices to stabilize the respiratory volume in most subjects while a 30-minute rest period is sufficient for practically all subjects. These periods of rest agree substantially with the findings of Benedict and Crofts (2) according to which a period of 30 minutes is long enough to stabilize oxygen consumption. However, our work also indicates that the minute respiratory volume under these standardized conditions is greater than the minute volumes under the fasting conditions which are routine in the measurement of basal oxygen consumption (2). Since previous observers have reported rest periods as long as 20 or 30 minutes, other factors must have caused the respiratory volumes to rise so much higher than they did in our experiments. These factors may have been: a greater resistance in the circuit, increased dead air space in the apparatus, and, in some cases, failure to reduce the volume to standard conditions of temperature and pressure.

SUMMARY

Continuous collections of expired air were made in a group of 31 young adult males immediately after they had assumed the supine posture, and were continued for 30 minutes. An average decrease in respiratory volume of approximately 40 per cent during the first 10 minutes and 10 per cent during the next 10 minutes was found. No significant decrease occurred between the twentieth and thirtieth minutes of rest. No evidence was obtained with successive testing that the rate of stabilization of the respiratory volume increased, although individual differences were noted. No significant alteration in respiration resulted from the application of a Siebe-Gorman half-mask.

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EFFECT OF OXYGEN TENSION OF INSPIRED AIR ON THE RESPIRATORY RESPONSE OF NORMAL SUBJECTS TO CARBON DIOXIDE[1]

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Opinions have differed widely concerning the influence of the alveolar oxygen tension on the respiratory response to carbon dioxide. Vernon (15) believed that high oxygen tensions decreased the respiratory response to carbon dioxide. Lindhard (9) and Hasselbalch and Lindhard (7) held that the respiratory center became more sensitive to carbon dioxide as the oxygen tension decreased. Campbell, Douglas, Haldane and Hobson (3) maintained that the alveolar oxygen tension could be varied within wide limits without sensibly affecting the respiratory response to carbon dioxide. The work of Eastman (5) and Selladurai and Wright (12) showed that the respiratory response to carbon dioxide is decreased in states of anoxemia.

The study of the relation of high oxygen tensions to the stimulating effects of carbon dioxide has received little attention. In fact, most of the investigations on carbon dioxide hyperpnea have been carried out without regard to the concentration of oxygen inhaled, except that in most instances anoxemia was avoided by using an "excess" of oxygen. To our knowledge Yamada (16) was the first to recognize that the inhalation of carbon dioxide-oxygen mixtures caused a greater respiratory increment than did the inhalation of carbon dioxide mixed with air. His experimental technique consisted of the measurements of alveolar carbon dioxide tensions. Davies, Brow and Binger (4) reported similar results but since "in some cases the percentage increase differed but little from the mean deviation," they were unwilling to lay any stress on their findings and felt that the matter could be decided definitely only "through statistical methods based upon a large amount of data collected under carefully

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standardized conditions." In our own work an attempt has been made to clarify the effects of breathing several concentrations of carbon dioxide in pure oxygen and in 21 per cent oxygen by carefully controlled experiments with a larger number of subjects.

EXPERIMENTAL. Twenty-three male college students, ranging in age from 18 to 28 years, served as subjects. The number of subjects used for each concentration of carbon dioxide may be seen in table 2. Each subject was tested twice with a given carbon dioxide concentration in the presence of both 21 per cent oxygen and high oxygen. Each experiment consisted of 1, a period of 30 minutes during which the expired air was measured while the subject breathed outdoor air; 2, the test period during which the gas mixtures were breathed (this period was long enough for the respiratory volume to become stabilized), and 3, an after-period of 20

TABLE 1

Mean per cent increase in respiratory volume

	TEST I				TEST II				AVERAGE OF TEST I + II			
	Mn	σ_d	σ_{Mn}	C.R.	Mn	σ_d	σ_{Mn}	C.R.	Mn	σ_d	σ_{Mn}	C.R.
100% O_2	13.7	15.2	3.6	3.8	12.4	8.9	2.1	5.9	13.3	11.4	2.7	5.0
1% CO_2 in air	11.8	11.1	2.7	4.4	16.2	8.2	2.0	8.2	14.0	7.6	1.8	7.5
1% CO_2 in O_2	27.6	14.5	3.5	7.8	28.8	14.2	3.5	8.3	28.4	13.9	3.5	8.2
2% CO_2 in air	30.9	15.2	4.4	7.1	37.2	16.1	4.6	8.0	34.2	13.2	3.8	8.9
2% CO_2 in O_2	50.0	18.4	5.3	9.4	54.8	24.2	7.0	7.8	52.8	19.7	5.7	9.3
4% CO_2 in air	97.1	35.8	8.7	11.2	105.4	41.5	10.4	10.2	101.3	36.5	9.4	10.7
4% CO_2 in O_2	122.2	47.1	11.4	10.7	124.6	57.5	14.4	8.7	121.1	45.4	11.7	10.3

minutes during which the subject again breathed outdoor air. Each subject was tested at a uniform time of day and had not eaten for one hour prior to each test. A Siebe-Gorman half-mask was adjusted to each subject and the expired air was measured over a period of 20 minutes while the subject was in the supine position in order to assure stable values of respiratory volume (13). The expired air was collected in a pair of recording spirometers of the Tissot type, each with a capacity of 9.19 liters (S.T.P.). These operated continuously in alternation and recorded electrically when each tank was filled. Egg shell valves (floating on mercury and opened by a pressure of 1.5 mm. of water) separated the inspired and expired air. After four preliminary trial runs, each on a different day, the experimental series was begun. Each subject was tested with only one mixture on any single day, but the tests with a similar mixture were repeated on a succeeding day.

In order to eliminate any effect of practice, the test series was counter-

balanced by having one-half the subjects breathe the carbon dioxide-air mixtures first and the other half the carbon dioxide-oxygen mixture first.

The gas mixtures were obtained in four 6000-liter high pressure tanks from which a pair of Tissot spirometers, each with a capacity of 60 liters,

TABLE 2

Effect of O_2 content on increment in respiratory volume resulting from increasing CO_2 in inspired air

Percentage increment in respiratory volume (based on average resting respiratory volume for each subject during fore-period)

SUBJECT NUMBER	1% CO_2			2% CO_2			4% CO_2		
	In 21% O_2	In 99% O_2	Diff.	In 21% O_2	In 98% O_2	Diff.	In 21% O_2	In 96% O_2	Diff.
1	12	61	49						
2	19	42	23						
3	12	16	4						
4	19	37	18						
5	20	23	3						
6	11	15	4	37	41	4			
7	8	30	22	34	49	15			
8	10	23	13	36	49	13	74	88	14
9	4	14	10	38	42	4	146	181	35
10	12	20	8	18	44	26	89	109	20
11	31	31	0	44	65	21	140	201	61
12	13	38	25	37	54	17	68	109	41
13	16	19	3	38	57	19			
14	21	35	14	22	71	49	108	202	94
15	9	23	14	24	42	18	80	90	10
16	12	32	20	41	74	33	129	163	34
17	13	24	11	42	45	3	107	118	11
18							86	113	27
19							97	106	9
20							63	99	36
21							90	96	6
22							93	120	27
23							109	132	23
Mean....	14.2	28.4	14.2	34.3	52.8	18.5	98.6	128.5	29.9
$\sigma_{Mn.\ diff}$...			3.0			4.1			6.3
C.R.....			4.7			4.5			4.7

was filled. The use of these large Tissot spirometers allowed the gas mixtures to come to the same temperature and pressure as the air in the room before being respired. One of the large spirometers was filled from the storage tanks while the subject breathed the mixture from the other. The intake valves were arranged so that the mixtures could be connected

to the inspiratory circuit without the knowledge of the subject. The carbon dioxide content of the gas mixtures from the high pressure tanks and from the large Tissot spirometers was measured before each separate test and was found to vary less than ± 0.04 per cent from the purported value.

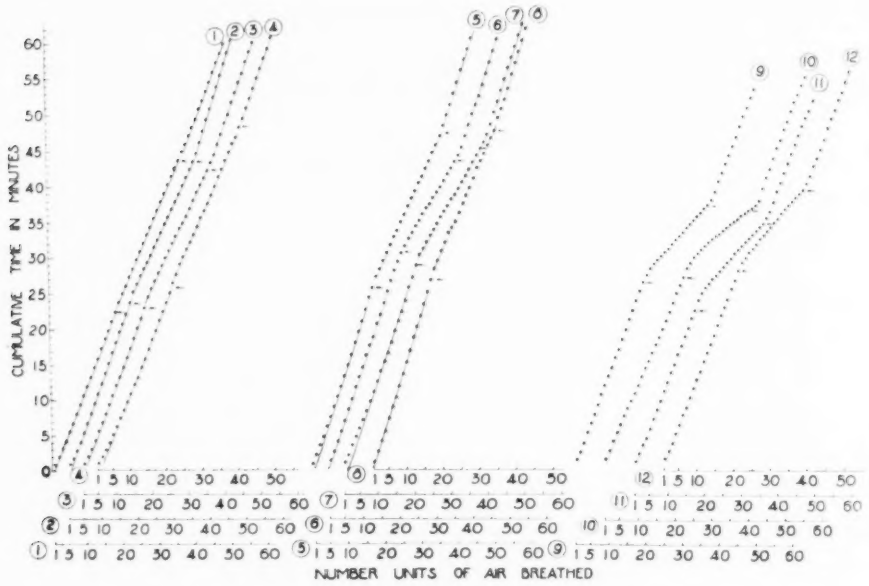

Fig. 1. Effect of alterations in O_2 tension on increase in respiratory volume produced by low concentrations of CO_2 in inspired air in normal male. Ordinate-cumulative time in minutes required to expire equal units of air (9.1 l.). CO_2-O_2 mixtures administered over intervals between arrows. All 12 experiments on single subject age 23. Height 176.6. Weight 77.4. Curves *1* and *4* are duplicate experiments in which a mixture of 1 per cent CO_2, 21 per cent O_2, and 78 per cent N_2 was administered. In curves *2* and *3* a mixture of 1 per cent CO_2, 99 per cent O_2 was administered. In curves *5* and *8* a mixture of 2 per cent CO_2, 21 per cent O_2 and 77 per cent N_2 was administered. In curves *6* and *7* a mixture of 2 per cent CO_2 and 98 per cent O_2 was administered. In curves *9* and *12* a mixture of 4 per cent CO_2, 21 per cent O_2 and 75 per cent N_2 was administered. In curves *10* and *11* a mixture of 4 per cent CO_2 and 96 per cent O_2 was administered. Experiments were run in the temporal order as numbered.

ANALYSIS OF DATA AND RESULTS. Respiratory volumes were computed by dividing the volume of expired air in each 9.19 liter tank (corrected to 0°C. and 760 mm. pressure) by the time required to fill each tank, and were recorded as volumes in liters per minute or as volumes in liters per square

meter per minute. In all the experiments the resting level was determined from an average of at least 10 observations following the 20-minute rest period. In order to decide which points should be included in the determination of the resting level, graphs were constructed (see fig. 1) in which cumulative time was plotted as the ordinate and the number of tanks filled with expired air was plotted as the abscissa.[2] Straight lines were fitted by free-hand methods to the experimental points so plotted. As can be seen in figure 1, these points form a straight line when the respiratory volume is constant. Increases in respiratory volume result in a decrease in the slope of the line. Average values were computed using only points after a linear trend had been established. The resting level was used as the value from which all deviations caused by breathing the carbon dioxide mixtures were computed. Table 1 is a summary of average results from all the subjects calculated in per cent, together with the critical ratios (computed as ratio of the mean difference between resting respiratory volume and stimulated volume divided by the standard error of the difference). The increments in table 1 are based on the averages of two tests for each subject, with the baseline calculated on the fore-period.

Table 1 indicates that carbon dioxide mixed with 21 per cent oxygen causes a significant increase in the respiratory volume in all concentrations used. One per cent carbon dioxide causes a mean increase of approximately 14 per cent, while 2 per cent carbon dioxide causes an increase of about 34 per cent. We found, as have others, that 4 per cent carbon dioxide doubles the respiratory volume. On the other hand, when the same concentrations of carbon dioxide are mixed with pure oxygen, the increments are much greater. Thus, 1 per cent carbon dioxide in oxygen increases the respiratory volume almost as much as does 2 per cent carbon dioxide in air.

Since each subject breathed a given percentage of carbon dioxide in 21 per cent oxygen and also the same percentage of carbon dioxide mixed with pure oxygen, the significance of the difference in respiratory response to carbon dioxide under the two conditions was tested by the method of differences, as summarized in table 2. This table shows that the response to all concentrations of carbon dioxide mixed with pure oxygen is significantly greater than to the same concentrations of carbon dioxide mixed with 21 per cent oxygen, since the critical ratios are 4.7, 4.5 and 4.7 for 1, 2 and 4 per cent carbon dioxide.

Discussion. A possible explanation of our results is that in the presence of a high oxygen tension in the respiratory center the sensitivity of these cells to the normal stimulus of increase in hydrogen ion concentration may

[2] This method of plotting was utilized because fewer calculations were required and because this method tends to minimize the effects of chance fluctuations.

be enhanced so that respiration is increased without a measurable rise in the hydrogen ion concentration or in carbon dioxide of the center. It is true that if 13.3 per cent (the average increment in respiratory volume produced by breathing 100 per cent oxygen) is deducted from the values obtained when carbon dioxide mixed with pure oxygen is inspired (table 2), the discrepancy is 0.9 per cent (14.2 − (28.4 − 13.3)) when breathing 1 per cent carbon dioxide; 5.2 per cent (34.3 − (52.8 − 13.3)) when breathing 2 per cent carbon dioxide; and 16.6 per cent (98.6 − (128.5 − 13.3)) when breathing 4 per cent carbon dioxide. If these increasing differences were statistically significant, it would prove that the respiratory center is more sensitive to a given concentration of carbon dioxide when the oxygen tension is increased. However, the differences, although suggestive, are not statistically significant.

Since it has been shown previously that a significant rise in respiratory volume results from breathing 100 per cent oxygen (14) we are forced to conclude that the results observed represent only an additive effect of increased O_2 tension and increased CO_2 tension. Since the differential effect does not disappear with breathing 4 per cent CO_2 we doubt whether it can be attributed to a reduction in blood flow in the brain which Lennox and Gibbs (8) and other investigators (10, 11) have found in humans and mammals breathing pure oxygen.

The rise in CO_2 tension of the tissues of animals breathing pure oxygen reported by Campbell (2), as well as the rise in CO_2 tension of both arterial and venous blood in animals breathing pure O_2 at 4 atmospheres' pressure reported by Behnke, Shaw et al. (1), lend support to the thesis originally proposed by Gesell (6) that in the presence of high oxygen tension the transport of carbon dioxide from the tissues might be interfered with because the amount of reduced hemoglobin available to transport a given amount of CO_2 would be reduced as a result of the increased amount of oxygen carried in physical solutions.

Practically, the conclusion to be drawn from our work is that a given concentration of carbon dioxide should be mixed with oxygen rather than with air to produce a maximal degree of hyperpnea. Conversely, (as concluded from the work of others), in states of anoxemia oxygen should be used in high concentration in order to enhance the respiratory response to either the carbon dioxide present or to the carbon dioxide that may be added.

SUMMARY. A group of 31 adult males has been studied. Each subject rested for 30 minutes in order to stabilize the respiratory volumes and then breathed (through a Siebe-Gorman half-mask) a mixture of 1 per cent carbon dioxide and 21 per cent oxygen for a period of 8 to 15 minutes, after which time the respiratory volume was again stable but at a higher level. This experiment was repeated with 1 per cent carbon dioxide and 99 per

cent oxygen. In all the experiments the average increment in the respiratory volume was greater when the subject breathed 1 per cent carbon dioxide in 99 per cent oxygen than when he breathed 1 per cent carbon dioxide in 21 per cent oxygen. Similar results were obtained when 2 and 4 per cent carbon dioxide were mixed with oxygen. In some subjects the difference in respiratory response was great enough to be of clinical importance and should be considered when carbon dioxide is administered for therapeutic purposes.

CONCLUSIONS

1. Data are shown which give the average effect of 1, 2 and 4 per cent carbon dioxide on the respiration of normal adult males.

2. Normal subjects respond with a greater increment in respiratory volume to carbon dioxide (in concentrations of 1, 2 and 4 per cent) mixed with pure oxygen than to the same concentrations of carbon dioxide mixed with air.

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PRESSOR RESPONSES FOLLOWING SHORT, COMPLETE RENAL ISCHEMIA: CHARACTERISTICS, MECHANISM, SPECIFICITY FOR KIDNEY

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That an elevation of blood pressure follows the restoration of renal circulation after periods of complete arrest has been reported by Katzenstein (1905), Dicker (1937, 1938), and Taquini (1938, 1939, 1940). In a preliminary publication Collins and Hamilton (1940) confirmed the results of Taquini, consistently obtaining elevations of blood pressure after $5\frac{1}{2}$ to $6\frac{1}{2}$ hours of complete, bilateral renal ischemia. At the same time Friedberg, Landowne, and Rodbard (1940) reported similar experiments, but obtained definite elevations of blood pressure in only 8 of 19 experiments. Later Prinzmetal, Lewis, and Leo (1940) uniformly observed pressor responses upon reëstablishing the circulation through one kidney after 4 to 6 hours of complete bilateral ischemia.

While extensive studies of chronic partial ischemia have been made, no comparable investigations exist for temporary complete ischemia. The latter may prove a valuable tool in the investigation of renal hypertension, and warrants a more detailed study. We have, therefore, considered the following phases of this problem: short periods of complete ischemia, unilateral ischemia, characteristics of pressor response, rôle of nervous mechanisms, effects of previous adrenalectomy and splenectomy, and ischemia of other organs.

METHODS. Dogs were anesthetized with chloralose except in 2 experiments where sodium pentobarbital was used. Through a mid-line abdominal incision all collateral circulation was destroyed by separating the kidneys from surrounding peritoneum and fat and by tearing visible blood vessels on the ureters. The artery and vein of each kidney were occluded by a special screw clamp provided with a sleeve extension, which passed through an abdominal stab wound. After closure of the abdomen, the clamps could be manipulated by the sleeve extensions without disturbance to the animal. Renal circulation was always examined at the end of the experiment, and was found to be reëstablished in all cases. Mean arterial blood pressure was recorded by a mercury manometer, usually from the femoral artery.

RESULTS. *Complete bilateral ischemia lasting 6 to 7 hours.* When the clamps, occluding the vessels of the 2 kidneys, were opened simultaneously after periods of complete ischemia similar to those used by Taquini (1938, 1939, 1940), elevations of blood pressure were consistently obtained (table 1).

TABLE 1

Periods of 6 to 7 hours of complete, bilateral renal ischemia

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3	EXPERIMENT 4*	EXPERIMENT 5*
Length of ischemia *hrs.*	6½	6½	7	7	6½
Original level of blood pressure *mm. Hg*	132	146	112	123	116
Maximum elevation of blood pressure *mm. Hg*	48	20	50	34	26
Elevation at end of experiment *mm. Hg*	38	18	38	32	24
Time between unclamping and end of experiment *min.*	24	27	16	21	15

* The animal was anesthetized with sodium pentobarbital.

TABLE 2

Shorter periods of complete, bilateral renal ischemia

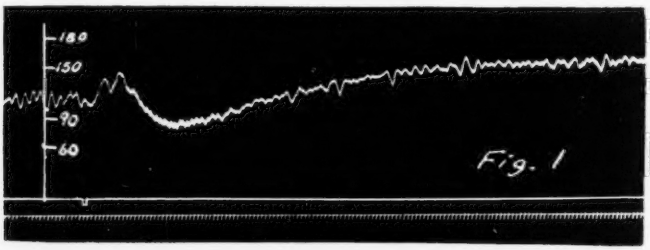
	EXPERIMENT 6	EXPERIMENT 7	EXPERIMENT 8	EXPERIMENT 9	EXPERIMENT 10	EXPERIMENT 11	EXPERIMENT 12	EXPERIMENT 13*	EXPERIMENT 14
Length of ischemia *hrs.*	3	3¼	3½	2¼	2	1	½	½	½
Original level of blood pressure *mm. Hg*	140	152	109	84	118	114	120	58	127
Maximum elevation of blood pressure *mm. Hg*	24	16	44	34	28	32	22	33	30
Elevation at end of experiment *mm. Hg*	24	6	44	31	28	32	14	24	30
Time between unclamping and end of experiment *min.*	49	18	36	25	19	46	24	65	9

* Blood pressure was recorded from the carotid artery.

Shorter periods of complete bilateral ischemia. The next series of experiments involved periods of ischemia shorter than those studied by other investigators. Pressor responses were uniformly obtained even with periods as brief as ½ hour (table 2, figs. 1 and 2 B). The length of the period of ischemia exerted only a slight effect on the magnitude of the response (tables 1 and 2). As the time was shortened, there was less damage of the kidney as evidenced by more normal gross appearance after

restoration of circulation. These organs, therefore, resemble the partially ischemic kidney (which may show no demonstrable damage) more closely than do those studied by previous investigators, using longer periods.

Characteristics of the pressor response. In many cases the pressor response consisted solely of a gradual elevation of blood pressure, beginning as a rule almost immediately after opening the clamps. In some experiments, however, this gradual rise was preceded by preliminary fluctuations.



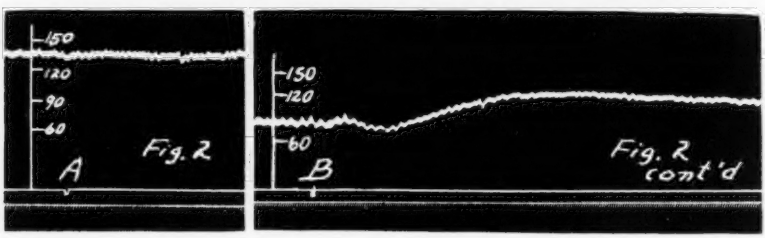


Fig. 1. Dog. 8. Restoration of circulation after 3½ hours of complete, bilateral renal ischemia. The clamps were opened at the signal.

Fig. 2. Control experiment. The clamps were applied to both pairs of renal vessels, previously ligated with linen thread, together with the intact, accompanying nerve plexuses. At A the clamps were opened, 1½ hours after their closure. The clamps were then retightened, and the linen ligatures were removed. At B, 45 minutes after A, the clamps were opened again.

In the figures the first line is the femoral blood pressure, the second is the base and signal line, and the third is a time line of 4 second intervals.

In most cases they consisted of a rapid rise followed by a fall to a level usually, but not always, below the original pressure (fig. 1). A nervous mechanism is not involved as experiments to be described later showed that these preliminary fluctuations were not produced by control manipulations or sham opening of the clamps and were present after denervation of the kidney and destruction of the cord combined with vagotomy. Furthermore, they are not dependent upon the presence of the adrenals or spleen, since they occurred after excision of these organs.

The blood pressure reached its maximum height 2 to 9 minutes after unclamping. The longer delays were usually associated with marked preliminary variations. The pressor response was prolonged (tables 1 and 2). In some of the subsequent experiments the blood pressure was recorded for an hour or more after the release of the clamps, and it did not fall to its original level in this time.

When all the experiments were analyzed, the magnitude of the response was found to be independent of the initial value of the blood pressure. In some of the animals, in which much of the spinal cord was destroyed, poor and atypical rises occurred when the blood pressure was low and continuously falling.

Complete unilateral ischemia. In 7 experiments the pressor responses from one kidney were studied; the second kidney was intact in 4 cases, and clamped in the other 3. The periods of ischemia varied from $1\frac{1}{2}$ to $8\frac{1}{4}$ hours, but most of them were less than $3\frac{1}{2}$ hours. In all instances typical rises of blood pressure (16 to 34 mm. Hg) occurred when the circulation through the single kidney was restored. Numerical values for elevations of blood pressure always refer to the maximum height of the prolonged rise, and not to the preliminary fluctuations.

Absence of a nervous mechanism. Experiments were next performed to determine what rôle, if any, nervous factors play in the response.

1. *Manipulation of the clamps.* In many of the experiments the clamps were either rapidly closed and opened or purposely pulled and twisted to a degree calculated to produce an amount of stimulation equal to that involved in opening the clamps; no significant changes of blood pressure resulted.

2. *Sham opening of the clamps.* Three control experiments, similar to those of Taquini (1938, 1939, 1940), were performed. After collateral circulation had been destroyed, clamps were applied to both pairs of renal vessels, previously ligated with linen thread, together with the intact accompanying nerves. When the clamps were opened $1\frac{1}{2}$ to 2 hours later, no change of blood pressure occurred (fig. 2 A). The experiments were continued by retightening the clamps and removing the ligatures. When the clamps were now opened, the usual rise of blood pressure occurred (fig. 2 B).

3. *Complete ischemia of denervated kidneys.* In 4 dogs, after separation of the kidneys from surrounding tissues, the ureters and the entire lengths of the renal vessels were mechanically stripped of nerves and generously painted with phenol solution. Following denervation the kidneys were subjected to about 2 hours of complete ischemia. Pressor responses occurred upon restoration of circulation (24, 30, 44, and 66 mm. Hg).

4. *Complete ischemia after cord destruction and vagotomy.* In 4 dogs most or all of the cord below the cervical segments was destroyed by a

heavy hooked wire, inserted into the spinal canal through a laminectomy. The extent of complete destruction, ascertained by opening the spinal canal at the end of the experiment, was as follows: dog 1—all below the first thoracic roots, dog 2—portion between the first thoracic and fifth lumbar roots, dog 3—all below the fifth cervical roots, dog 4—all below the sixth cervical roots. After the kidneys were rendered ischemic, the vagi were sectioned. The duration of the initial ischemia varied from 1 to 1½ hours. In 3 of the animals the kidneys were subjected to a second period of ischemia (⅔ to 1½ hrs.). In 4 of the 7 unclampings prolonged elevations of blood pressure of 14 to 30 mm. Hg resulted, preceded with one exception by unusually prominent preliminary variations. In the other 3 unclampings, where the blood pressure was low and continuously falling, the pressor responses were small or poorly maintained.

The preceding series of experiments indicate that the pressor response is of non-nervous origin; consequently a humoral mechanism must be involved. This conclusion coincides with that drawn by Taquini principally from different types of experiments. In addition, the last group of experiments indicates that the renal pressor material does not act exclusively, if at all, through the central nervous system since the thoracolumbar cord, from which the sympathetic vasoconstrictor fibers arise, was largely or entirely destroyed.

Complete ischemia of structures other than the kidney. It is important to determine whether the phenomenon is a special property of renal tissue. Other structures were, therefore, studied.

1. *Leg.* In 3 dogs the hind leg was completely isolated, except for the blood vessels, at a point just above the knee joint. The vessels were then occluded for 1½ to 2 hours, during which time the leg was kept at about body temperature. When the clamp was opened, there was no significant change in blood pressure in any of the 3 animals. Circulation was found to be intact at the conclusion of the experiments.

2. *Liver.* In 3 dogs the circulation through the narrow pedicle-like root of the left division of the central lobe of the liver was completely obstructed for about 2 hours. When the clamp was released, a fall of blood pressure occurred in one animal and no change in 2. At the end of the experiments examination showed that circulation had been restored. The weight of the hepatic tissue subjected to ischemia varied from 63 to 119 grams.

Thus, as far as leg or liver is concerned, the pressor phenomenon is specific for the kidney.

Complete, bilateral renal ischemia after adrenalectomy. After recent bilateral adrenalectomy characteristic pressor responses were obtained as follows: dog 1, 18 mm. Hg (2½ hrs.); dog 2, 36 mm. Hg (2 hrs.), 18 mm. Hg (additional 2 hrs.); dog 3, 37 mm. Hg (3⅙ hrs.).

Complete renal ischemia after splenectomy. In 3 recently splenectomized dogs, periods of complete bilateral ischemia of $1\frac{7}{8}$, $1\frac{1}{10}$, and $2\frac{5}{8}$ hours gave elevations of blood pressure of 32, 36, and 46 mm. Hg respectively.

Discussion. The conclusion that the elevation of blood pressure following restoration of circulation through the completely ischemic kidney is due to the liberation of pressor material from this organ is supported by other observations in the literature. These observations will be briefly summarized.

Taquini (1938, 1939, 1940) obtained pressor responses by transplantation of completely ischemic kidneys (about 6 hrs.) or by injection of their venous blood. Non-ischemic kidneys gave negative results. Using the Läwen-Trendelenberg preparation he found vasoconstrictor properties in venous plasma from completely ischemic kidneys, but not from normal kidneys or completely ischemic spleens. Mason and Rozzell (1939), however, found the Läwen-Trendelenberg preparation unsatisfactory for the study of vascular effects of dog sera.

Pressor responses have been obtained from perfusates of kidneys rendered completely ischemic for 24 hours (Dicker, 1937, 1938) and 4 to 6 hours (Prinzmetal, Lewis and Leo, 1940). Williams and Grossman (1938) found pressor substances in renal perfusates. Although the kidneys were not purposely made ischemic, a period of a few minutes to 2 hours or more elapsed before the perfusions were performed. These authors also found 2 active substances in such perfusates, one resembling epinephrine, the other renin; this claim may bear a relationship to the preliminary variations obtained in our experiments. All the above investigators failed to find pressor activity in perfusates of completely ischemic organs other than the kidney. This finding agrees with our results on leg and liver.

While it appears likely that the pressor material involved in persistent hypertension from partial renal ischemia is closely related to the agent involved in complete ischemia, the relationship has by no means been clarified. However, it is significant that Loesch (1933) was able to produce moderate persistent hypertension by repeated brief occlusions of the renal vessels and ureters.

SUMMARY AND CONCLUSIONS

Taquini's findings are confirmed; intervals of complete, bilateral renal ischemia of 6 to 7 hours invariably result in elevations of blood pressure when circulation is restored.

Briefer intervals of complete renal ischemia, even those as short as $\frac{1}{2}$ hour, consistently give pressor effects. The magnitude of the responses is only slightly less than that from longer periods.

The characteristics of the response are as follows: the blood pressure

rises gradually, reaching a maximum 2 to 9 minutes after the release of the occluding clamps; the elevation is prolonged; in some cases the gradual rise is preceded by preliminary changes, consisting usually of a relatively rapid rise followed by a fall; these preliminary variations are not due to stimulation incident to opening the clamps; the gradual prolonged response is independent of the height of the initial blood pressure.

Occlusion and subsequent restoration of the blood supply of one kidney, either with the other intact or clamped, is followed by a prolonged elevation of blood pressure.

A nervous mechanism is not involved in these responses. This conclusion is based on 4 groups of experiments: 1, manipulation of the clamps; 2, opening of the clamps with the vessels of the kidney ligated and the accompanying nerve plexuses intact; 3, complete ischemia with the kidneys and their vessels denervated; 4, complete ischemia with the thoracolumbar cord destroyed and the vagi cut. The responses must, therefore, be due to pressor material from the ischemic kidney. This material does not exert its pressor action solely, if at all, through the central nervous system.

If the circulation to the leg or to a portion of the liver is arrested for about 2 hours, no elevation of blood pressure occurs when the circulation is restored.

Neither recent adrenalectomy nor recent splenectomy prevents an elevation of blood pressure following restoration of renal circulation after complete bilateral ischemia.

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INTRALUMEN PRESSURES OF THE DIGESTIVE TRACT, ESPECIALLY THE PYLORIC REGION[1]

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The literature on the digestive tract contains numerous references to the intralumen pressures of the gut. The limitations of the methods commonly employed in such investigations should be appreciated, for in general they are physically inadequate to supply quantitative values. The fluid or semifluid gastro-intestinal contents will tend to move from a region of higher to a region of lower intralumen pressure; thus observations, usually roentgenographic, of direction and rate of translocation of contents afford a rough suggestion of pressures.

Records obtained by the balloon-water manometer technic have frequently been interpreted in terms of intralumen pressure, but the objections to this system have been so universally unappreciated that a critical analysis is desirable. Certain objections are referable to the water manometer. Since it has a high volume-pressure coefficient it cannot yield quantitative values. This was illustrated as follows: With a 3 x 10 cm. balloon in a pressure chamber, but closed off from its water manometer, introduction of 5.5 cc. of air into the chamber produced a pressure of 10 cm. of water. On restoring connection between the balloon and water manometer, the pressure fell to 5.2 cm. An additional 5 cc. of air in the chamber was required to restore the pressure to 10 cm. Also, unless the balloon contains a large volume, displacement of its entire contents may be insufficient to record the maximum pressure applied to the balloon. However, a balloon of large volume constitutes an undesirable foreign body and it records from a large region, not from a point.

The pressure, P, developed at any instant within a cavity whose size is changing is related to the time rate of volume change $\left(\frac{dv}{dt}\right)$ and to the resistance offered to the escape of contents, R, as shown by the formula $P = \frac{dv}{dt}R$. A pressure device having a large volume-pressure ratio pro-

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vides an abnormal channel for escape of material from the cavity in which pressure is to be measured. By reducing R, an error in pressure is introduced which bears no constant relation to the naturally occurring pressure. This abnormal channel for escape of material alters both physically and physiologically the efficiency of the cavity as a pressure creating mechanism. Substitution of a bromoform manometer, a mercury manometer, a recording tambour, or an optical manometer progressively decreases the magnitude of the error due to volume change.

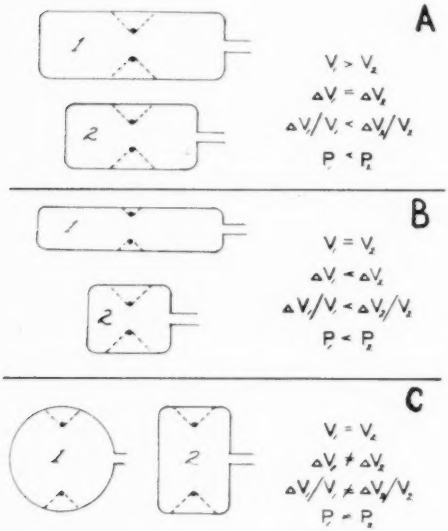

Fig. 1. The pressure produced by a ring slipped over a balloon will vary according to the volume, size and shape of the balloon. With each pair of balloons the constricting rings have the same diameter. A. The balloons have the same shape and diameter but not the same volume. B. The balloons have the same shape and volume but not the same diameter. C. The balloons have the same volume and diameter but not the same shape.

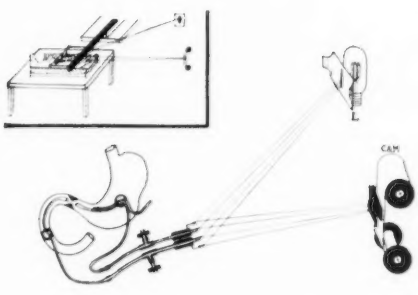

Fig. 2. Method of optical registration of pressure in the pyloric antrum and duodenal bulb.

The balloon has certain inherent objections when used for the quantitative measurement of pressure. When the pressure is applied by a fluid medium, uniformly to the entire balloon surface, as in a pressure chamber or the "encased balloon" method (discussed subsequently), these objec-

tions are minimal. When the balloon is distorted into a dumb-bell shape by a constricting ring, such as by the direct contact of a peristaltic contraction of the gut, pressures will be dependent to an unpredictable extent on the volume, size and shape of the balloon (fig. 1). Also, if the constricting ring causes a decrease in balloon volume, artificial pressures, not existing in the gut lumen, may develop in the balloon. These pressures will be inversely related to the ease with which the rubber stretches. We have recorded pressures from a small balloon (having an effective volume of 0.3 cc.) in the gut 2 to 3 times as great as from a physically accurate recording device fastened adjacent to it.

Technic. Although the principles of accurate pressure registration have been well established by Frank (1), they have not previously been applied to the determination of gastro-intestinal pressures. We have applied these principles in constructing an optical manometer which consists of a brass tube with 1.5 mm. bore connected to the portion of the gut to be studied by lead and rubber tubing having an internal diameter of 1 mm. The total length was about 45 cm. The recording end of the manometer was closed with a rubber membrane supporting a small mirror chip which received light from a slit lamp and reflected the beam to a photokymograph 2.5 meters from the mirror (fig. 2).

The maximum rate of motility in the pyloric sphincter region may be taken as 30 contractions per minute and the frequency of the fastest significant component as 15 times this or 450 per minute (7.5 per sec.). This manometer is adequate to record such pressure changes without distortion since it has a higher vibration frequency; 75 per second when recording from air, 20 per second when recording from water or gastric juice and 15 per second from cornmeal mush. The vibration frequency of an ordinary water manometer recording from air is 1 to 2 per second. The coefficient of damping of our manometer is greater than 0.9 (i.e., the oscillations are practically critically damped) so it is capable of faithfully recording sinusoidal pressure waves of nearly 15 per second from cornmeal mush. Application of Fourier's method of harmonic analysis to the curve shows that the error in the registration of maximal values is less than 1.3 per cent. The sensitivity of the manometer is altered at times to meet special requirements by varying the thickness and tension of the rubber membrane but usually a deflection on the photokymograph of about 2 cm. is obtained for 10 cm. of water pressure and the volume change in the apparatus is 0.1 cc. for 50 cm. of water pressure.

Open tube method. The "open tube method" was employed in most of our studies. In this method, the rubber tube in the gut terminates in a hollow cylindrical tip made of cellulose acetate which is firm enough to retain its shape, but soft enough to be practically non-irritating to adjacent tissue. The overall length of the tip is 2 cm., the free end has an internal

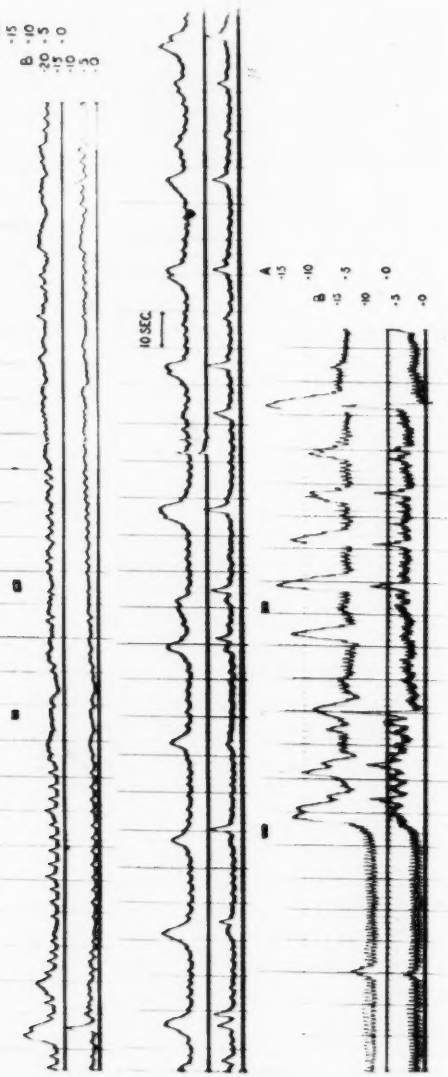

Fig. 3. Effect on antral and bulbar pressures of feeding by mouth (2 upper records) or by cannula (lower records). Interval of feeding indicated by the heavy horizontal marks.

diameter of 4 mm. and the end attached to the tubing is turned down to 1 mm. In determining the pressures within the pyloric antrum and duodenal bulb, two open cellulose acetate tips are drawn into the spaces to lie 3 mm. proximal and distal to the sphincter (fig. 2). In normal trained dogs provided with permanent metal cannulae by the technic of Meschan and Quigley (2), several hundred experiments have been performed. At the onset of the experiment food has been withheld for 24 hours. The animals are placed in the dorsal position in a comfortable hammock where they remain without restraining bands. When the recording tubes are connected to optical manometers as described, the intralumen pressure occurring at the mouth of each plastic tip is measured.

The records are not affected by pressure of the gut wall on the tube, and the volume change with pressure is not significant. The small tube in the gut has essentially no distending effect on the gut wall and the effect of the recording tip as a foreign body appears to be minimal.

Encased balloon method. We also recorded the intralumen pressure with a balloon 1 cm. long and 0.5 cm. diameter and containing when in use 0.3 cc. air. This is attached by a rubber tube to an optical manometer. This balloon is encased in a metal capsule having numerous perforations. When records are made simultaneously from the encased balloon and from an open tube lying adjacent to it, the phasic pressure changes are es entially identical. In general, results obtained from the open tube method and the encased balloon are identical except that the open tube records from the *point* at the mouth of the tube in the gut, while the encased balloon records from the larger space or *region* of the gut in which it lies. With the encased balloon, changes in temperature or position of the tubes may create false pressures (not true with the open tube method) and basal pressures are recorded less accurately. Furthermore, the encased balloon is a more objectionable foreign body than the open-tube. Passage of gastric contents into the encased balloon is impossible but such material may enter the open tube (it enters the plastic tip about 6 mm. for a pressure of 50 cm. water and a pressure greater than 150 cm. is necessary to force material into the narrow bore end). Possible obstruction of the open tube from this source is minimized by routinely blowing 1 cc. of air through the tube at intervals and always when the changes characteristic of plugging appear.

Results and interpretations. *The empty antrum and bulb form multiple transitory closed cavities.* It was shown in several ways that the pressure changes measured with the stomach and duodenum empty were from closed cavities even when the gastric and duodenal lumen communicated with the exterior by the open cannulae 7 to 9 cm. from the recording tips. In a series of experiments, the metal cannulae were covered with "alba stone cement" which adhered firmly to the skin and precluded leakage between the cannulae and the abdominal wall. The continuous mass

of cement also covered the mouth of the cannulae through which the recording tubes passed and thus escape of gut contents through these channels was prevented. The records obtained using these precautions were identical in all respects with those obtained when the cannulae were simply plugged with vaselined cotton and therefore the cannulae were sealed by the latter method in the majority of the experiments.

Rubber tubes having a 4 mm. bore were introduced into the gut; one tube 4 cm. orad from the antral pressure recorder, the second 4 cm. aborad to the bulbar recorder. The opposite end of both tubes extended to the exterior. The records were not modified by opening or closing these tubes. However, when the tubes to the exterior were fastened by the side of the antral recording tip or the tubes were placed in tandem so the open ends were 3 mm. apart, the antral pressure sharply fell to zero when the tubes to the exterior were opened.

When phasic pressures were taken simultaneously from two recording tips, one in the prepyloric region of a fasting dog and the other 1.5 cm. proximal to it, the records evidently were from different cavities. The proximal curve began and terminated earlier and the peak usually was slightly lower than from the distal cavity. On the other hand, two records made from the same cavity (open tubes 2.5 cm. apart in either end of the perforated metal capsule without a balloon) were identical. If the antrum contains water the tendency to form completely separate cavities decreases and records made from two portions of the antrum show more resemblance to each other.

Basal and phasic pressures. The basal pressures within the antrum and bulb are partly extrinsic, partly intrinsic in origin. As in an inanimate object, the extrinsic portion is related primarily to the intra-abdominal pressure in the vicinity of the structure under observation. With the animal at rest, the intra-abdominal pressure will depend on the postural contractions of the abdominal muscles and the diaphragm, but since the abdomen resembles an inverted glass of water, the pressure is lowest at the top of the cavity and increases progressively at lower levels. The extrinsic pressure also is modified by the weight of organs lying on the structure studied, the traction of tissue dependent from it and the weight of the upper wall of the structure. The intrinsic pressure results from the tonic contraction of the antrum and bulb respectively. The gut exhibits basal pressure when no contractions are in the vicinity of the recording tip, for repeated fluoroscopic observations of animals given barium sulfate either with a meal or as an "outliner" showed the region to be quiescent while displaying only basal pressure.

With the animal lying in the dorsal position, basal antral and bulbar pressures during periods of fasting vary between -3 and $+3$ cm. of water. Superimposed on these are pressure elevations of about 2 cm. with each

inspiration and additional small oscillations due to the pulse are observed. Sub-atmospheric pressures, occasionally reaching −8 cm., are most frequent and most marked in the bulb. Usually negative pressures persist for about 1 to 2 seconds but they may continue for 8 to 10 seconds. The pressure for 10 to 15 minutes is sometimes sub-atmospheric as much of the time as it exceeds atmospheric. Transient negative pressure waves are especially frequent immediately after a phasic wave. They are rarely obtained immediately preceding a phasic wave when they might be anticipated if a wave of relaxation precedes a wave of contraction as implied by Bayliss and Starling's Law of the Intestine. The enlargement of the gut which precedes a peristaltic wave is ascribed by Alvarez (3) and Henderson (4) to the distending pressure of advancing gut contents. Our results fail to support this suggestion for an elevation of pressure rarely precedes a phasic wave in the regions we studied. Wilson and Irving (5) reported negative pressures in the fundus of the full or empty stomach, but they employed the balloon method.

If intra-abdominal pressure in the sphincter region is sub-atmospheric, a completely relaxed antrum and bulb should also show negative pressures, while higher basal pressures would result from various degrees of antral or bulbar tonus. In preliminary studies using optical registration, we have repeatedly recorded sub-atmospheric intra-abdominal pressures. Negative intra-abdominal pressures have also been reported by Keppich (6), Reprev (7), Wagoner (8), Overholt (9), Lam (10) and others. However, sub-atmospheric pressures of the gut may not result exclusively from negative intra-abdominal pressures.

Basal antral pressure during fasting usually exceeds bulbar pressure by 1 to 2 cm.; thus factors interfering with the free passage of material between the two cavities must be present. This conclusion probably does not require active contraction of the pyloric sphincter, for the anatomical conformation of the sphincter region, its rough surface and mucous plugs may provide sufficient resistance. A higher basal pressure in the antrum than in the bulb may indicate a greater state of tonic activity in the former region.

Phasic augmentation of pressure superimposed on the basal pressure occurs during fasting both in the antrum and bulb approximately 3 to 4 times a minute. Typically, the antral wave begins slightly before the bulbar wave; they reach a maximum simultaneously and the bulb regains the basal level in advance of the antrum. Although deviations from this pattern are sometimes encountered, the two waves are so closely related and simultaneous fluoroscopic observations show antral, sphincter and bulbar contractions in such regular sequence as to preclude a chance relation. A passage of influences from antrum to bulb is strongly indicated. This observation is in accord with the demonstration by Meschan and

Quigley of the progressive character of the contraction process in the antrum, sphincter and bulb. On the contrary, Joseph and Meltzer (11) report that after each antral contraction the duodenum stops its rhythmic contraction and loses its tone, a manifestation of the Law of Contrary Innervation. Alvarez and Mahoney (12) emphasize a blocking of peristaltic waves at the sphincter; Thomas and Crider (13) find the gastric influence on the duodenum is predominantly inhibitory and Barclay (14) reports that antral peristalsis stops dead at the sphincter and does not pass into the duodenum. The influence transmitted from the antrum to the bulb to produce the sequential relationship noted by us may be either nervous or mechanical. It need not be dependent on the filling of the bulb by antral contents for the relation holds during fasting when nothing is being evacuated from the stomach.

Antral phasic pressure waves persist for 5 to 7 seconds, bulbar waves for 2 to 4 seconds. Maximal pressures are essentially the same in the antrum and bulb and usually range between 15 and 30 cm., but occasionally pressures of 60 to 90 are obtained. Employing the water manometer or tambour technic von Pfüngen (15) recorded a pressure of 162 cm. in the prepyloric region, Moritz (16) observed cyclic prepyloric pressures of 20 to 30 cm., or occasionally 40 to 60 cm., while Sick (17) obtained pressures of 25 to 42 cm. near the pylorus. In fasting animals we find periods of phasic pressure changes continuing for 5 to 10 minutes alternate with periods of quiescence lasting for approximately 60 minutes. The periodic character resembles ordinary hunger contractions recorded from the body of the stomach rather than the continuous activity recorded by Meschan and Quigley (2) from the sphincter region. Alvarez (3), Barclay (14), Wilson and Irving (5), and Goette and Grosser (18) state that roentgen cinematographic and roentgenographic or balloon studies on man and animals show that peristaltic contractions never close the antrum from the orad portion of the stomach and therefore peristalsis is incapable of elevating pressure in the antral lumen. Our results show this description is generally applicable to the proximal antrum but when the wave involves the prepyloric region, a closed cavity develops and the pressure rises. Also, reference to the formula $P = \frac{dv}{dt} R$ shows that if the contraction is rapid, so the rate of volume change is high, pressure may develop even though the lumen is not completely occluded by the wave.

Fluoroscopic studies in our animals when given $BaSO_4$ show that antral and bulbar phasic pressure changes failed to occur without associated contractions. We conclude that antral phasic waves develop when a contraction wave decreases the volume of the cavity occupied by the recording tip and interferes with orad escape of contents at a time when sphincter closure or bulbar resistance obstructs aborad passage of material. In the bulb, phasic pressure develops when the bulb contracts while the

sphincter, orad and the duodenal tone aborad offer resistance to the movement of bulbar contents. A further indication that the sphincter closes and separates the two cavities at the time phasic pressures develop arises from the observation that antral and bulbar pressure changes are rarely identical in magnitude or in time of onset and termination. However, when the pyloric sphincter of fasting animals is propped open with a spool-shaped object having a 5 mm. bore, thus providing free communication between the two cavities, basal pressures in the antrum and bulb agree and phasic pressures coincide in all respects.

Caution must be exercised in expressing phasic pressure changes in terms of peristaltic contractions, for, although they are related, the relationship is not simple. The slope of a pressure curve does not necessarily indicate the rate of gut contraction or relaxation, e.g., a slight relaxation may suffice to connect a cavity of high pressure with one of low pressure, resulting in a rapid fall of pressure in the former cavity, although the gut muscle continues to relax long after the intralumen pressure approaches the basal level.

Ingestion of food. When 150 cc. of thin, strained mush (cornmeal and meat) is administered orally while a series of phasic antral and bulbar pressure variations is in progress, they are replaced by irregular fluctuations of 0.5 cm. of water magnitude occurring 30 to 50 times per minute. Fluoroscopic observations show that peristaltic contractions are inhibited. At the cessation of deglutition the normal antral and bulbar pressure pattern and the peristaltic contractions are promptly regained. If phasic pressure changes and peristaltic contractions are absent preceding the ingestion of food, they usually appear shortly afterwards. If food is simply placed near the animal's nose, phasic changes also disappear but reappear within two minutes following removal of the olfactory stimulus. This influence from eating or smelling food is equally effective in the full or empty stomach. It occurs in vagotomized animals and thus the vagi need not be involved in the reflex.

If the mush is introduced *via* the gastric cannula without attracting the dog's attention the phasic changes do not disappear but, on the contrary, are usually exaggerated. This occurs despite the fact that the rate of introduction is only $\frac{1}{2}$ to $\frac{1}{3}$ as rapid as when the meal is fed by mouth. Whether the food is given orally or by cannula, within a few minutes after feeding, the gradient of pressure from antrum to bulb increases, the phasic changes become more frequent, more uniform and usually of greater magnitude, and this type of activity persists for an hour or more. The records closely resemble those obtained from the pyloric region of fasting or fed dogs by the tandem balloon technic of Meschan and Quigley (2) and tend to confirm the impression that even in the fasting dog the tandem balloon, perhaps because of its distending action, records the "fed" type of motility.

When food is administered orally or by cannula, the basal pressures are

elevated and the gradient of pressure from antrum to bulb is increased. Subatmospheric pressures are still obtained occasionally, especially following a phasic wave. Cannula feeding usually produces an immediate rise in basal pressure but with oral feeding it may be delayed for a few minutes. After 150 cc. of mush, antral pressure reaches 4 to 6 cm., bulbar pressure 1 to 3 cm., but with 1 liter of mush, antral pressure may be 7 to 8 cm. and bulbar about 4 cm. Identical results were obtained in double vagotomized animals. The elevation of antral and bulbar basal pressure after feeding may be due either to a general increase in the tone of the antrum and bulb or to an increase in the quantity of material entering these cavities. Against the latter suggestion is the fact that the typical increase occurs even on those occasions when not enough of the meal enters the antrum and bulb for some minutes after feeding to permit their visualization. Subsequently, the quantity entering the antrum at one time is moderate. The delayed rise in pressure after oral feeding and the immediate response to cannula feeding must involve a reflex producing a difference in tonus development.

Employing dogs several months after double vagotomy we obtained pressure records indistinguishable as regards basal pressure, frequency and magnitude of phasic changes from those of normal dogs except the former showed a slower onset of phasic changes following the ingestion of food. The literature describing the gastric effects of vagus section is contradictory, but these observations are in accord with the report of McCrea, McSwiney and Stoppford (19) of normal gastric motility after vagotomy. However, they do not support the observation of Meek and Herrin (20) who reported a permanent loss of gastric tonus following this operation.

We are grateful for technical aid in this investigation from Miss M. R. Read and Mr. F. J. Rack.

SUMMARY

A critical analysis of the ordinary balloon-water manometer method emphasizes its inadequacy for the registration of gastro-intestinal pressures. Many objections are related to the high volume-pressure ratio, but in addition the recorded pressures will vary with the size, shape and volume of the balloon. Pressures are accurately measured from an open tip in the gut and an optical manometer for registration. The basal pressure of the pyloric antrum usually exceeds the basal pressure in the duodenal bulb and both rise moderately when food enters the stomach. Sub-atmospheric pressures are common in both regions. Periodically, phasic pressure changes amounting to about 30 cm. of water develop in both regions. The phasic pressure changes of the bulb are generally closely related to those in the antrum. They are produced respectively by bulbar or antral contractions. Swallowing or smelling food produces a transient inhibition

of these pressure changes even in vagotomized animals. After feeding, this inhibition is quickly supplanted by phasic changes more uniform, more persistent and frequently of greater magnitude than preceding the feeding.

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